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# Petfood: physico-chemical characteristics and functional properties of meat by-products and mechanically separated chicken (MSC) in a high-moisture model system

José Antonio Rivera  
*Iowa State University*

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Petfood: physico-chemical characteristics and functional properties  
of meat by-products and mechanically separated chicken  
(MSC) in a high-moisture model system

by

José Antonio Rivera

A dissertation submitted to the graduate faculty  
in partial fulfillment of the requirement for the degree of  
DOCTOR OF PHILOSOPHY

Major: Meat Science

Major Professor: Joseph Sebranek

Iowa State University

Ames, Iowa

1998

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*To mankind*

*To the essence of the universe*

*To my brothers for their love and friendship*

*To my dear parents for their guidance and sacrifice*

*To my grandfather for his endurance over 90 years of struggle*

*To my extended family for showing me the complexity of society and human nature*

*"Supina cibum animalia sua paravit sed spiritualis panis filii sui superandus est"*

**(Camilo P. Garcia, 1998)**

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## ABSTRACT

Two studies were conducted to determine the physico-chemical characteristics and functional properties of selected pork and poultry by-products used in the formulation of high-moisture petfood. Mechanically separated chicken (MSC) served as control. Correlations were established among meat by-product and MSC functional properties and their individual physico-chemical characteristics.

In the first study, pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and MSC were evaluated for proximate composition, protein distribution and connective tissue. Proximate composition varied among meat by-products and MSC. Pork by-products contained the highest level of crude protein ( $p < 0.05$ ). Low levels of high ionic strength soluble (HIS) protein were obtained among meat by-products and MSC relative to levels of low ionic strength soluble (LIS) proteins and insoluble (IN) proteins. Pork lungs and chicken viscera contained the highest levels of IN proteins ( $p < 0.05$ ). Total collagen values were positively correlated to IN proteins, intramuscular collagen (IMC) and elastin. No Types I and III collagen were detected by SDS-PAGE (12.5%) for the different meat by-products.

In the second study, the contributions to water retention capacity (% WRC) and texture changes were determined for pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and MSC as affected by pH and various salts in a high-moisture model system. The % WRC increased for meat by-products and MSC with increased pH (4.5 - 6.8). Pork lungs and MSC had the highest % WRC ( $p < 0.05$ ) among the meat by-products. Meat by-product % WRC was not significantly ( $p > 0.05$ ) affected by salt (2%), phosphate (0.3%) or NaOH (0.075%). Chicken viscera had the lowest ( $p < 0.05$ ) mean texture measurements among the meat by-products and MSC. Strong negative correlations ( $p < 0.05$ ) were obtained for texture with total collagen, soluble collagen and high ionic strength soluble (HIS) proteins.

No beneficial change in %WRC was obtained for meat by-products with the addition of salt, phosphate or sodium hydroxide. Likewise, addition of the same inorganic salts had little effect on texture of meat by-products (except for lungs) as measured by Instron values. Chicken viscera was the least functional meat by-product. Conversely, MSC was positively improved ( $p < 0.05$ ) with the addition of salt and sodium hydroxide. Additional research is warranted to determine if texture and %WRC are significantly improved by the addition of MSC to mixtures of pork and poultry by-products in combination with inorganic salts.

## CHAPTER 1. GENERAL INTRODUCTION

### Introduction

Petfood manufacture for cats and dogs has developed into a large industry over the last 30 years (Rainbird, 1988). Petfood is available in three basic forms: canned, semi-moist and dry (Anonymous, 1982). Canned petfood, which may contain up to 78% moisture, is generally preferred by most cats and dogs (Corbin, 1978). High-quality nutritionally-balanced canned petfood is usually higher in animal proteins and in animal fat but lower in carbohydrates than dry or semi-moist petfood (Kelly, 1996). The petfood industry relies heavily on by-products of the meat packing and poultry processing industries as sources of animal protein (Morris, 1990).

Despite the wide use of meat by-products by the petfood industry, there have been very few attempts to characterize them. Information on the functional and physico-chemical properties of by-product proteins is extremely limited (Nuckles et al., 1990). Most of the published research has concentrated on composition and quantitation of different protein fractions (myofibrillar, sarcoplasmic and stromal) of selected beef and pork by-products (Oliveros et al., 1982; Ibarra et al., 1985; Kim et al., 1991). Unfortunately, in these studies, no attempts were made, other than collagen determination, to further characterize the connective tissue (stromal fraction).

A central hypothesis to this work was that connective tissue plays an important role in the manufacture of canned petfood. Meat by-products contain varying amounts of connective tissue (Bailey and Light, 1989). High levels of sarcoplasmic and stromal proteins in meat by-products compared to mechanically separated chicken (MSC) and lean meat have been reported (Rivera et al., 1998; Kim et al., 1991; Nuckles et al., 1990). Connective tissue, or more specifically collagen, shrinks and becomes granular at 60°C during thermal processing

and melts to form gelatin when heated to 75-80°C (Whiting, 1988). Collagen's rate of gelatinization increases with temperature, occurring very rapidly at 125°C (Whiting, 1989). Thus, significant changes in texture and water holding capacity can be expected in canned petfood containing high levels of collagen.

Therefore, information on the functional properties of meat by-products, as it pertains to canned petfood, is needed. A rational utilization of meat by-products by the petfood industry will depend largely on this information. Studies covered by this dissertation aimed to characterize selected meat by-products with particular emphasis on the stromal protein fraction. Also, experiments were undertaken to better understand the effect of environmental factors (pH, ionic strength) and processing conditions (temperature) on final texture and water holding capacity of selected meat by-products. Lastly, an effort was made to relate functional properties of meat by-products to their individual physico-chemical characteristics.

### **Dissertation Organization**

This dissertation consists of a general introduction (Chapter 1), a literature review (Chapter 2), two articles to be submitted for publication (Chapters 3 and 4) and a general summary (Chapter 5). Each paper consists of an abstract, introduction, materials and methods, results and discussion, conclusions and references.

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## CHAPTER 2. LITERATURE REVIEW

*"In our role to be wanted and accepted we turn to animals- thus fulfilling a basic need".*

Corbin, 1978

### **Petfood**

The term petfood in the market place refers commonly to dog and cat food. However, the overall petfood industry caters to other species such as birds, reptiles, rodents and serpents (Miller, 1996). In this particular dissertation, the term "petfood industry" will refer solely to dog and cat food.

### **Types of Petfoods**

The manufacture of foods specially prepared for cats and dogs has developed into a large industry over the last 30 years (Rainbird, 1988). In the US, 90% of dogs and cats receive most of their nourishment from commercial petfood (Rodebush, 1993). Commercial foods for the companion animals are available in three basic forms: canned, semi-moist and dry (Anonymous, 1982). They differ primarily according to means of processing and preservation. They also differ in composition, i.e. ingredients and chemical analysis (Kronfeld, 1984). A more complete description of these three types of petfood is given in section "Petfood Manufacture".

### **Pets and Humans**

There are few formal scientific studies of why people own pets or of the variety of needs which are satisfied through pet ownership (Feldmann, 1978), though pet ownership is and has

been widespread. Historically, Roman archeological remains show evidence of the human bond with pets by such things as dog and cat footprints in rooftiles and bricks (Miller, 1996). Throughout history and throughout the world people have adopted pets (Feldmann, 1978). Companionship and affection are the reasons more often given for owning a pet (Anonymous, 1982). According to the market analyst, Euromonitor, reasons such as reduced birth rates, changing family structures, aging populations and a growing love of animals have contributed to a worldwide rise in pet ownership (Anonymous, 1996). A pet is a faithful, intimate, noncompetitive and nonjudgmental friend who may provide comfort and security against intruders and loneliness (Fieldmann, 1978).

### **The Worldwide Petfood Market**

The market analyst, Euromonitor, estimates that the world pet population has reached nearly 500 million animals (98 million cats and 92 million dogs). According to the same market analyst, the world market for petfood was worth US\$ 22.6 billion in 1995 and was estimated to grow to US\$ 24.7 billion by 1997. Growth factors for the industry include rising pet populations (particularly in emerging markets), changes by consumers from using kitchen scraps to buying prepared foods and major manufacturers using more sophisticated marketing (Anonymous, 1996).

### **The US Petfood Market**

Maxwell (1997) reported that, in 1996, there were 55.8 million dogs in 37.6% of US households. In 1997, the dog population was estimated in 56.6 million in 38.0% of US households. Likewise, in 1997, the cat population was estimated in 69.6 million in 33.7% of US households, up from the 67.9 million in 33.4% of US households.

In 1996, the US petfood industry reported retail sales of US\$ 9.4 billion. In 1997, sales were estimated in US\$ 10.1 billion. Dog food sales alone in 1997 were estimated to account for US\$ 5.7 billion. Similarly, in 1997, cat food sales were estimated to account for US \$ 4.4 billion (Maxwell, 1997).

### **Petfood Development**

Some petfoods are formulated to supply sufficient amounts of all essential nutrients. Others are intended to form only part of the ration, i.e. to be used as supplements or treats (Kronfeld, 1984). Most commercial petfoods provide adequate nutrition to support excellent maintenance, growth and reproduction (Fahey et al., 1997).

### **Nutritional Needs**

Nutrition is of paramount importance and is the parameter most closely evaluated in the initial stages of any petfood development program (Booth, 1976). Proteins, fats and carbohydrates are the three major groups of nutrients in any petfood. They provide companion animals with the basic monomers (amino acids, fatty acids and sugars) required to support both maintenance and any additional physiological (growth, gestation or lactation) needs (Hussein et al., 1997).

The National Research Council (NRC) of the US National Academy of Sciences has published tables of the recommended dietary nutrient levels in diets for dogs and cats. These figures are designed to provide the nutrients required for the entire life cycle (Grøndale and Hedhammar; Blaza and Loveridge, 1984).

The Petfoods Subcommittee of the Association of American Feed Control Officials (AAFCO) published a set of protocols to be followed in collecting animal test data to support nutritional advertising claims (i.e., "complete", "complete for growth" and "complete for normal adult maintenance"). Rainbird (1988) defined complete foods as those containing all the nutrients required by the dog or cat for that particular stage of the life cycle for which they are sold (i.e. for adults, for puppies). Products not intended as a sole diet are categorized as supplements (Smith and Norvell, 1975).

## **Meat Ingredients**

### **Fresh and /or Frozen Animal By-Products**

Meat ingredients may be included in pet diets for specific marketing-related reasons, as well as for nutritional and functional purposes (Fahey et al., 1997). Different sources of protein are normally used to formulate petfoods depending upon the type desired. The petfood industry relies heavily on by-products of the meat packing and poultry processing industries as sources of protein (Morris, 1990). Animal-based ingredients are rich in protein and contain appreciable amounts of fat. This protein is primarily high quality and can provide companion animals with the amino acids required for maintenance, growth, gestation and lactation (Fahey et al. 1997). In addition to supplying protein, animal-based ingredients, are good sources of calcium, phosphorus and other nutrients (Long et al., 1975). In spite of being expensive when compared to vegetable proteins, quality animal protein sources are, and most likely will continue to be preferred, especially in cat diets (Hussein et al., 1997). Cats are true carnivores and must have foods of animal origin in their diet (Rainbird, 1988).

The US meat industry produces over 30 billion pounds of animal by-products per year (Phillips, 1994). Meat by-products are commonly classified as edible or inedible (Oliveros et al., 1982). The yield of edible products from a meat animal is typically 45-60% of live weight,

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dependent on the type of species and the extent to which edible by-products are recovered (Jobling, 1986). Poultry and red meat processors freeze slaughter by-products into blocks for shipping to petfood manufacturers. Upon arrival, the blocks pass into "breaker" machines in order to disintegrate and thaw the blocks into a mass or slurry that is used to make many types of petfood (Gill, 1989).

According to the AAFCO, meat by-products are defined as the non-rendered, clean, wholesome parts of the carcass of slaughtered mammals, such as lungs, liver, spleens, kidneys, brains, stomach and intestines (free of their contents). It does not include skin, horns, teeth and hooves. Blood and bone are considered as meat by-products (Hischke, 1975).

Blood comprises approximately 8-12% of the live weight of the meat animal according to the type of species and age. Blood is probably the largest single by-product (Jobling, 1986). It contains high levels of lysine and tryptophan (Phillips, 1986). However, blood proteins are not used extensively because of their effect on the appearance of the final product (Morris, 1990). Conversely, increasing quantities of fresh bone are being ground to a "bone emulsion" for inclusion in petfoods (Jobling, 1986).

Poultry by-products must consist of non-rendered clean parts of carcasses of slaughter poultry, such as head, feet and viscera. They must free from fecal content and foreign matter except in such trace amounts as might occur unavoidable in good factory practices (Hischke, 1975).

### **Rendered Animal By-Products**

Meat by-products are underutilized and low-priced because they are regarded as an inferior protein source for human food compared to skeletal muscle meat (Oliveros et al., 1982). A large portion of these by-products are converted into usable products by the rendering industry (Phillips, 1994). Raw material of animal origin that is not suitable for human consumption is processed in commercial processing plants to yield a large quantity of

animal protein meals for use by the animal feed industry (Fahey et al., 1997). The process fractionates raw material into water, fat and solids. The solid fraction is protein-rich and is typically processed into high quality protein meals that serve as an excellent source of dietary protein for dogs and cats (Hussein et al., 1997). Rendered petfood ingredients include meat and bone meal, poultry by-product meal, blood meal, feather meal, specialized protein blends, fish meal and animal fat (Phillips, 1994). Their chief use is as part of dry manufactured foods (Rainbird, 1988).

The following definition of the different rendered products was given by Phillips (1994).

#### ***Meat and Bone Meal***

Meat and bone meal consists of the protein residue and remaining bone after fat has been extracted in the normal rendering process. Raw materials include: packing house by-products (subcutaneous fat, organ fats, offal and bones), boning house material, butcher shop trimmings, restaurant greases and fallen animals.

#### ***Poultry By-Product Meal***

Poultry by-product meal consists of the ground rendered parts of the carcasses of slaughtered poultry, such as heads, feet, undeveloped eggs and intestines, which is exclusive of feathers, except in such trace amounts as might occur unavoidably in good manufacturing practices.

#### ***Blood Meal***

Blood meal is a finely ground protein residue derived from raw beef blood. Spray drying methods are preferred since they produce blood meal with a much higher lysine content. Blood meal excludes all extraneous material such as hair, stomach belchings and urine, except in such traces as might occur unavoidably in good manufacturing practices.

***Feather Meal***

Hydrolyzed feather meal is derived by pressure cooking the clean, undecomposed slaughter poultry feathers. The protein content in feather meal runs in the low 80% range. High quality feather meal should have a low fat content (5% or less).

***Specialized Protein Blends***

These are blends of blood meal, feather meal and meat and bone meal in various percentages. The blends are created to meet specific protein and amino acid requirements for ruminant and monogastric animals.

***Fish Meal***

Fish meal is manufactured by a similar process to the meat and bone meal process. Fish meal protein, because of its high biological value, finds great acceptance by the petfood industry, especially in cat diets. Fish meal contains 60 to 72% crude protein, 6 to 12% moisture, 2 to 14% fat and 10 to 20% ash (Hussein et al., 1997).

***Animal Fat***

Generally referred to as fats, they are produced in conjunction with high protein meals and include rendered fats from beef or pork by-products. This is mainly packing house offal or supermarket trimmings from the packaging of meats (Rouse et al., 1987).

The generic terms meat, bone and fish meals are being replaced by distinguishing terms of the animal's origin. This is due in part to market forces that show preferences for beef, lamb, venison, salmon, tuna, catfish, etc. (Miller, 1996).



### **Mechanically Separated Animal Tissues**

Mechanically separated beef and mechanically separated chicken are also good sources of protein for the petfood industry. Mechanically separated meat is produced by utilizing specially designed equipment that crushes the bone and also separates bone, cartilage, ligaments and tendons from soft tissues (Judge et al., 1989). These products are typically produced, packed and frozen in 50 lb blocks by the packer-renderers (Miller, 1989). The standard of identity for these products was listed in the CFR 319.5 as follows: "Mechanically separated (species) is any finely comminuted product resulting from the mechanical separation and removal of most of the bone from attached skeletal muscle of livestock carcasses and parts of carcasses. Such product shall have a protein content of not less than 14 percent and a fat content of not more than 30 percent. At least 98 percent of the bone particles present in such product shall have a maximum size no greater than 0.5 millimeter".

### **Non-Meat Ingredients**

In the meat industry, a variety of non-meat products referred to as extenders, binders or fillers, are used in the processing of meat products (Judge et al., 1989). The purpose of non-meat ingredients (i.e. soy proteins) is to lend nutritional value, to aid in structure and to contribute desirable flavor and odor qualities (Levinson, 1975).

There has been a growing use of non-meat proteins (particularly those from milk and soy) in meat products as additives, extenders and even as complete replacements of meat proteins (Jobling, 1986). However, in the petfood industry, the selection and usage level of the less expensive plant protein sources are limited by other factors. Hussein et al. (1997) explained that besides the palatability requirements and nutritional value, secondary compounds in plant protein sources are a concern. According to the same researchers, secondary compounds (e.g. lectins, tannins, trypsin inhibitors and complex oligosaccharides) are undesirable in petfoods.

especially in premium diets. Premium diets have a focus on quality and are manufactured from high quality raw materials. They are high in digestibility and palatability and are assessed for nutritional adequacy by feeding trials (Kelly, 1996).

Cereal grains are valuable dietary ingredients in petfoods as they contribute both a source of available energy and protein to the diet (Morris, 1990). The amount and type of cereal used will depend on nutrients needed by the animal and the petfood product characteristics desired (Long et al., 1975). Cereal grains represent 30 to 60% of dry matter in companion animal diets (Hussein et al., 1997).

Other sources of protein such as leaf and single cell proteins, have been used as feed for farm animals. However, currently they are not competitive on a price basis with the oilseed proteins (Morris, 1990). Nonetheless, proteins of fungal and microbial origin are expected to become the next generation of additives to or substitutes for meat (Jobling, 1986).

### **Petfood Additives**

Manufacturers use a variety of food additives for petfood production. Food additives are defined as substances purposely incorporated in foods to provide desirable characteristics. This includes color, flavor, texture, stability or resistance to spoilage (Roudebush, 1993). The following description of food additives was given by Roudebush (1993).

- Antioxidant preservatives frequently used in petfoods include tocopherols, citric acid, ascorbates, ethoxyquin, propyl gallate, tertiary butylhydroquinone and butylated hydroxyanisole.
- Antimicrobial preservatives such as sodium propionate, potassium sorbate, sodium nitrite, etc., are used in soft-moist petfoods and treats because of the high moisture content of the soft moist products.
- Humectants such as propylene glycol, sorbitol, etc., reduce water activity, prevent loss of water after processing and add nutritional value to soft-moist petfoods.

- Natural and synthetic colors are added to enhance consumer appeal or to prevent discoloration. These include nitrites, bisulfites and ascorbate.
- Natural and synthetic flavors may be added to provide flavor claims for petfoods. The flavor (i.e. digest, liver meal, monosodium glutamate) is included at 1 to 5% of the total product.
- Dry commercial petfoods are inherently less palatable than products with higher moisture contents. Most manufacturers use additives such as phosphoric acid, digests (i.e. hydrolyzed liver), spices, etc., to overcome this palatability gap.
- Gums (hydrocolloids), glycerine, glycerides and modified starch are used to prevent separation of ingredients and create the gravy or sauce portion of canned petfoods. These additives allow the creation of high-moisture foods that are highly palatable to the pet, but do not have an excess of free water.

### **Vitamins and Minerals**

Diets of companion animals are formulated to meet the known nutrient requirements and when necessary, are fortified with vitamins and minerals.

### **Petfood Evaluation**

In human societies, cultural factors provide a framework for identifying what is edible and inedible within that culture (Thorne, 1997). Pet owners tend to select petfoods to their own liking, that is to say, similar in appearance and consistency to what they put on their own table (Anonymous, 1987). Especially in city environments, dogs and cats exercise little choice as to what and how they are fed (Booth, 1976).

Acceptance of the food by the animal is also of paramount importance in food selection. Product testing is a key process to the development and improvement of petfoods and to the

understanding of the animal behavior underlying food selection (Thorne, 1997). The measure of feeding performance is commonly achieved through food intake using the assumption that a greater intake of food over another indicates higher palatability for that food (Thorne, 1997). Palatability can be thought of as being the sum of flavor, texture and color (Marshall, 1976).

The two-pan, free-choice method, the most common palatability test in the petfood industry, was described by Griffin (1996). Animals are offered free access to two pans, each containing a different diet in an amount greater than the animal would be expected to consume during the testing period. When there is more than a single test period, the left/right positions of the diets is alternated across periods. Result interpretation, as described by Owens (1982), is done using a statistical method that measures both the number of animals preferring a given ration as well as the degree of preference, as shown by the relative amounts of each diet consumed by each animal over the test period.

There appear to be very few odors, tastes or flavors which can be defined as inherently acceptable or unacceptable to cats or dogs (Thorne, 1997). Cats and dogs are essentially carnivores, thus, flavor research focuses on aroma of meats and offals that are specially acceptable to pet animals (Booth, 1976). Therefore, proteins of animal origin have a premium value (in addition to their amino acid profile) to the petfood industry (Morris, 1990).

Product formulation, size, shape, density and texture primarily determine palatability. Canned and soft-moist petfood products are more palatable than dry petfood, primarily because of their higher water content (Owens, 1982). Ingredients like whey and yeast, along with a coating of fat, may be added to dry petfood to increase palatability (Long et al., 1975). Spices, onion, garlic and extracts are frequently included in petfoods to enhance palatability (Roudebush, 1993).

## **Petfood Manufacture**

### **Canned Foods**

According to Hischke (1975), the canned petfood market can be segmented into the luxury-type products and the maintenance-type products. The basis of this segmentation is primarily ingredients, physical form and cost. The luxury types are high in meats and meat by-products and low in cereal grains. The maintenance types are a combination of both meat by-products and cereal grains. According to Corbin (1978) canned foods may contain up to 78% moisture.

The general manufacturing procedure was described by Hischke (1975) as consisting of a controlled size reduction of the meat and meat by-products (cubes, bits or chunks), mixing of the prepared meats and meat by-products with the remaining formulation (dry compounds, binders and processing aids), filling, closing and cooking.

### **Semi-Moist Foods**

Moisture content of semi-moist food is between 30 to 40% (Corbin, 1978). Semi-moist food types are available as snacks or treats for dogs and cats. They are presented as "minced pieces", burgers, balls or chunks (Kelly, 1996). Semi-moist foods can be made with a variety of ingredients including meat, meat by-products, soya or other vegetable-protein concentrates, cereals, fats and sugars (Rainbird, 1988). Humectants such as sugars, salts, glycerol are incorporated to reduce the water activity and to prevent bacterial growth. Further protection is provided by the use of preservatives or by the reduction of pH.

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## **Dry Foods**

Moisture content of dry foods is close to 10 % (Corbin, 1978). Dry foods have a long shelf-life because of their low water content (Rainbird, 1988). Dry foods for dogs and cats are usually in the form of extruded products; however, baked products are still available in the market for dogs (Kelly, 1996).

### **Extruded Products**

Manufacture of extruded products was described by Morris (1990). The process begins by mixing the ground dry ingredients (i.e. corn, wheat, milo and rice), a high-protein meal (i.e. meat, meat and bone meal, poultry by-product meal or corn gluten meal, soybean meal) and a vitamin / mineral premix. These ingredients then pass into a conditioner and then an extruder.

Extrusion cooking as described by Rokey (1994), converts cereal grain and protein blends into a dough. The starchy components gelatinize resulting in a substantial uptake of moisture and an increase in dough viscosity. During gelatinization, as explained by Gill (1990), the moisture holding capacity of cereals increases dramatically, resulting in expansion of the product. The type of expanded petfood desired will have some effect on ingredients used in the formula (Long et al., 1975). Protein constituents, as indicated by Rokey (1994) may impact elasticity and gas-holding properties that are characteristic of hydrated and developed glutinous doughs. Proteinaceous materials such as meat meal and fish meal, may contribute less to the adhesive and stretchable functional properties of the dough. The moist, cooked meal (Morris, 1990) exits from the extruder under pressure through a die and expands to produce a cooked product with a high volume: density ratio. Following extrusion (Long et al., 1975), petfoods are dried, cooled and fat applied to the outside to improve palatability.

Today, a vast range of extrusion equipment and techniques allows a bewildering array of shapes, textures and colors in a wide variety of products- from grain-based dry pellets and nuggets to meat or soy-based soft, moist petfoods (Gill, 1990).

### **Baked Products**

In the case of baked petfood products, the product is made into a dough, rolled into thin layers and then baked (Long et al., 1975). Some of these products are used for promoting dental health by physical abrasive action (Kelly, 1996).

### **Petfood Labeling**

In the US, petfood manufacturers must follow FDA rules for adequate manufacturing procedures, permitted ingredients and allowable claims (Phillips, 1990). Roudebush (1993) indicated that petfood additives must conform to the requirements in the Code of Federal Regulations, as food additives (21 CFR 573) or as ingredients generally recognized as safe (GRAS; 21 CFR 582). Petfood labels must be registered and approved (Phillips, 1990).

Requirements for petfoods labels were described by Phillips (1990):

- Products must be conspicuously identified as dog or cat food.
- Brand name must not be misleading as to content or nutritional properties.
- Chemical analysis must be guaranteed.
- Ingredient listing must be in descending order or preponderance.
- Evidence must be provided for any nutritional claim.

Currently, petfood labeling regulations allow for certain flexibility in petfood formulation. Fahey and Hussein (1997) contend that the way ingredients are currently presented can be misleading. For example, the ingredient list does not provide information about the quality of the dietary components. Also, the ingredient list can be deceptive because there is no

requirement for listing ingredients on a dry matter (DM) basis (not so much a problem for dry foods).

## **Meat Proteins**

### **Muscle Tissue**

Muscles can be classified as either striated or non-striated (Bechtel, 1986). Striated muscles exhibit regularly spaced transverse bands along the length of the cell and can be further subdivided into skeletal and cardiac muscle. Non-striated muscles are often referred as smooth muscles (Judge et al., 1989).

### **Skeletal Muscle**

Skeletal muscles are organs of the muscular system that are attached directly or indirectly to bones, ligaments, fascia, cartilage or skin (Judge et al., 1989). The muscles of the skeleton exist in a range of sizes and shapes depending on their function (Bailey and Light, 1989). Skeletal muscles are a very complex contractile system made up of cylindrical, multinucleated muscle fibers (cells) of varying lengths (Romans et al., 1985).

### **Skeletal Muscle Fiber**

The differentiated muscle cell in postnatal muscle is the muscle fiber, a highly specialized, long, cylindrical cell that can range in diameter from 10 to 100  $\mu\text{m}$  and in length from millimeters up to many centimeters (Allen, 1988). Muscle cell diameters vary with exercise, animal age, animal sex, nutritional state, breed, species and type of muscle (Bechtel, 1986). Muscle fibers do not generally extend the length of the entire muscle (Judge et al., 1989). The

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contractile structure of a muscle fiber is made up of the long, thin elements called myofibrils (Huxley, 1958).

### **Myofibrils**

Myofibrils are long, thin, cylindrical rods, usually 1-2  $\mu\text{m}$  diameter (Judge et al., 1989). The myofibril is an aggregation of 12 to 14 proteins into highly contractile threads that are insoluble at the ionic strength of the cytoplasm in muscle cells (Allen, 1988). A myofibril is made up of two kinds of myofilaments, one of which is twice as thick as the other (Huxley, 1958).

### **Myofilaments**

Myofilaments are commonly referred to as thick and thin filaments of the myofibril (Greaser et al., 1984; Judge et al., 1989). According to Allen (1988), thick filaments measure approximately 15 nm by 1,500 nm and thin filaments are roughly 6 nm by 1,000 nm. These myofilaments overlap somewhat and slide together to enable the muscle to contract (Romans et al., 1985).

### **Myofibrillar Proteins**

Proteins which compose the myofibrils (1 to 2  $\mu\text{m}$  in diameter) within the muscle fibers are collectively defined as the myofibrillar proteins (Asghar et al., 1985). Myofibrillar proteins constitute the proteins associated with thick and thin filaments and are referred to as salt soluble proteins (Judge et al., 1989). The salt-soluble myofibrillar proteins constitute between 50 to 55% of the total muscle protein content (Acton et al., 1983). Myofibrillar proteins are usually extracted from muscle with high ionic strength solutions, but once extracted some of them are soluble at low ionic strength (DeFreitas, 1994). Based on the physiological function in muscle, Asghar et al., (1985) classified myofibrillar proteins in contractile and regulatory proteins.

### ***Contractile Proteins***

The myofibrillar proteins, myosin and actin, which are directly involved in the contraction-relaxation cycle of the live muscle, are named contractile proteins.

#### **Myosin**

Myosin (actomyosin in the postrigor state) is the most abundant protein in meat and is also the dominant protein in the thick filaments of the myofibrils (Egelandsdal et al., 1993). Myosin accounts for approximately 43% of the total protein in mammalian and avian skeletal myofibrils (Khalili and Zarkadas, 1988). Myosin is a hexameric protein composed of four light chains and two heavy chains. Each of the heavy chains has a molecular weight of about 200 kDa, and the four light chains have molecular weights ranging from 15 to 27 kDa (Xiong, 1994). A review of different studies by DeFreitas (1994) found myosin as the most important component of the salt-soluble proteins involved in protein gelation. According to Egelandsdal et al. (1993), at pH 5.5, between 0.4 and 0.6 M NaCl would be needed to induce depolymerization of the thick filaments resulting in liberation of myosin and actomyosin. Myosin or actomyosin possess high water-binding capacity and viscosity (He, 1995). Myosin is also reported as having extensive emulsification capability resulting from protein-lipid and protein-protein interactions (Acton et al., 1983). Myosin has an isoelectric point of approximately 5.4 (Schmidt and Trout, 1982).

#### **Actin**

Actin is the major constituent of the thin myofilament and accounts for 22% of the myofibrillar protein (Asghar et al., 1985). In skeletal and cardiac muscles, actin exists as double helical filaments composed of polymerized globular monomers with a molecular weight of approximately 43 kDa (Xiong, 1994). Actin alone does not exhibit any binding properties (i.e. emulsifying capacity), but in the presence of myosin it reveals a synergistic

effect on binding, primarily due to the formation of actomyosin, a complex between actin and myosin (Asghar et al., 1985).

### ***Regulatory Proteins***

Myofibrillar proteins that are not directly involved in cross-bridge formation but play a role indirectly in the contraction-relaxation cycle, are called regulatory proteins. These proteins can be further divided into two groups; the major (tropomyosin and troponins) and the minor (M-protein, C-protein, F-protein, H-protein, X-protein, I-paramyosin, actinins,  $\alpha$ -actinin,  $\beta$ -actinin,  $\gamma$ -actinin, Eu-actinin and Z-protein) regulatory proteins. A review of several studies by DeFreitas (1994) found that the major regulatory proteins had no effect on the gel strength of actomyosin. However, Asghar et al. (1985) reported a significant increase in binding quality and water holding capacity by the addition of native tropomyosin to actomyosin system in the presence of pyrophosphate and  $MgCl_2$  when compared with tropomyosin-free systems.

### **Sarcoplasmic proteins**

Sarcoplasmic proteins in skeletal muscle consist of proteins that are soluble at low ionic strength ( $0.1\mu$ ), located in the sarcoplasm (muscle-cell cytoplasm) and account for approximately 30 to 34% of the total protein (DeFreitas, 1994). Sarcoplasmic proteins include myoglobin and enzymes associated with glycolysis, the tricarboxylic acid cycle and the electron transport chain (Judge et al., 1989). The content of myoglobin in skeletal muscle will vary depending on the metabolic profile of the muscle, animal species and age of the animal (Bechtel, 1986). Sarcoplasmic proteins are of relatively low molecular weight, globular or rod-shaped in conformation and have low viscosity (Asghar et al., 1985). They have isoelectric points between pH 6.0 and 7.0 (Schmidt and Trout, 1982). The sarcoplasmic

proteins are reported as having very low water-binding capacity and gel forming ability, but contribute a great deal to the meat color (He, 1995).

### **Smooth Muscle**

Non-striated or smooth muscle is composed of cellular units that are not subject to voluntary control (Bechtel, 1986). Smooth muscles are commonly referred to as visceral muscles and are found throughout the digestive and reproductive tract of animals. They are also present throughout the blood vessels, capillaries and arteries (Romans et al., 1985). Smooth muscle fibers vary in size and shape, depending on their location (Judge et al., 1989). Smooth muscle fibers, despite having about the same proportion of actin and myosin as skeletal muscle fibers, have a single nucleus and no striations along the fiber (Bailey and Light, 1989). Myofilaments of smooth muscle are less ordered than in skeletal muscle. The major contractile proteins in smooth muscle are myosin, actin, tropomyosin,  $\alpha$ -actinin and filamin while troponin is absent (Bechtel, 1986). Smooth muscle fibers form membrane-to-membrane contact bridges with neighboring fibers and these are supported by a delicate network of reticular fibers (Bailey and Light, 1989).

### **Non-Muscle Tissue**

Non-muscle animal tissues are referred to as offal. Variety meats or offals were listed by Romans et al. (1985) as consisting of the heart, tongue, liver, pancreas (sweetbreads), thymus (veal sweetbreads), kidney, spleen, brain and the walls of the stomach (tripe). With the exception of the brain, all these tissues contain varying amounts of connective tissue (Bailey and Light, 1989).

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## **Connective Tissue**

Muscle cells are mechanically attached to bones via complex mixture of macromolecules which are collectively referred to as connective tissue (Greaser, 1997). The three main roles of connective tissues are to give mechanical strength to organs, to provide framework for movement and to promote the right environment for cell growth and proliferation (Bailey and Light, 1989). Connective tissue proteins, also referred to as stromal proteins, constitute about 10 to 15% of the total muscle protein (Asghar et al., 1985). The physical properties of the various connective tissues are determined by their composition and specific macromolecular organization (Bailey and Light, 1989). Connective tissue is composed of an extracellular matrix whose major protein components are collagen, elastin and the proteoglycans (Jones, 1984).

## **Collagen**

Collagen, among the three major connective tissue protein groups, is the most important. A significant amount of collagen research has been conducted over the last fifty years (Jones, 1984; Bailey and Light, 1989). Collagen is the major connective tissue protein and accounts for about 30% of the total protein in the mammalian body (McCormick, 1989). Collagen fibrils are arranged in different ways, depending on the biological functions of the particular type of connective tissue (Jones, 1984). In skeletal muscle, three collagenous structures can be distinguished morphologically- endomysium, enclosing each muscle fiber, perimysium, surrounding bundles of these fibers, and epimysium, surrounding the whole muscle.

### **Tropocollagen**

The subunit of the collagen fiber is tropocollagen. Tropocollagen, which is a long rod-like molecule, 1.5 nm in diameter and 300 nm long, is a unique triple stranded helical structure stabilized by hydrogen bonds and intramolecular crosslinks (Jones, 1984). Tropocollagen is comprised of three polypeptide chains (called  $\alpha$ -chains) wound into a helix. According to Bailey and Light (1989), each  $\alpha$ -chain contains long sequences of repeating tripeptides based on the general structure Gly-X-Y (where X is commonly proline and Y can represent any amino acid but is often the modified hydroxyproline). Sims and Bailey (1981) indicated that the presence of the amino acid hydroxyproline in collagen (about 14%) is a unique feature because this amino acid occurs in only a few other proteins (i.e. elastin, 1.6%). Hydroxyproline is essentially peculiar to collagen and whose main function seems to be to stabilize the molecule and the fibril (Eyre, 1980).

### ***Types of Collagen***

The collagen molecule can be of homogeneous composition (identical  $\alpha$ -chains) or consist of combinations of different  $\alpha$ -chains (McCormick, 1989). There have been 19 different collagen types identified, each named by a Roman numeral in the order discovered (Greaser, 1997). Bailey (1989) classified the different types of collagens, based on the aggregation patterns, as fibrous, non-fibrous and filamentous collagens. Fibrous collagens (types I, II, III and minor collagens V and XI) self-assemble to form fibers possessing a characteristic band pattern (quarter staggered fashion) with a periodicity of 67 nm (Bailey, 1989). Type IV (non-fibrous), in contrast to fibril-forming types, assembles into a "chicken-wire" network type arrangement (Greaser, 1997). A number of recently identified minor collagens (types VI, VII, IX and X) possessing variable molecular lengths form a variety of filamentous structures (Bailey and Light, 1989). The different collagen types have different distribution in the connective tissue layers with type I dominating in the epimysium, type III being concentrated

in the perimysium and type IV being the major component in the endomysium / basal lamina (Sims and Bailey, 1981; Greaser, 1997). There are five types of collagen that are tissue-specific and are described as follows:

### **Type I collagen**

Type collagen I is found in the greatest amounts in the body and makes up the largest proportion in muscle (Eyre, 1980; Bailey and Light, 1989). Asghar et al. (1985) described the molecule of type I collagen as composed of two  $\alpha 1(I)$ -chains and one  $\alpha 2(I)$ -chain, whose structural form is written as  $[\alpha 1(I)_2\alpha 2(I)]$ . Type I collagen is found in all major connective tissues including skin, bone, tendon, dentine and all intra-organ connective tissues (Bailey and Light, 1989).

### **Type III collagen ("Reticulin")**

Asghar, (1985) described Type III collagen as consisting of three identical  $\alpha 1(III)$ -chains, whose structure is designated  $[\alpha 1(III)]_3$ . Type III collagen is found in embryonic tissue, scar tissue, skin, arteries, heart valve and many intra-organ connective tissues (Bailey and Light, 1989). Some researchers (Sims and Bailey, 1981; Asghar, 1985) have suggested that type III collagen and reticulin are the same. Reticulin, a mucoprotein, is believed to constitute the finest net of fibers in the endomysium layer of muscle (Asghar et al., 1985). Researchers Burson and Hunt (1986). found that type III collagen constitutes about one third of the total intramuscular collagen.

### **Other Collagen Types**

Type II collagen is usually associated in lesser amounts with Type I collagen (McCormick, 1989). Type II collagen is the predominant form in cartilage, while Types IV and V collagen are the main constituents of base membranes (Miller, 1987).

## **Elastin**

Elastin is a fibrous protein found in those tissues requiring a high degree of elasticity (Sims and Bailey, 1981). Elastin (Bailey and Light, 1989), is a major constituent of blood vessels and certain ligaments (40-60%), and other compliant tissues as lung and skin (2-5%). In skeletal muscle, its primary location is the epimysium and perimysium (Rowe, 1986). Generally, in muscle, elastin constitutes only a small proportion (<0.5%) of the overall content of connective tissue (Asghar et al., 1985). Elastin is regarded as having a rubber-like structure consisting of randomly coiled peptide chains cross-linked at intervals by stable chemical bonds (Sims and Bailey, 1981). The amino acid composition of elastin is unusual, containing about 40% glycine, plus proline and 40% hydrophobic amino acids (Bailey and Light, 1989). Elastin is a highly insoluble protein (in most reagents) due to the presence of a network of stable crosslinks (desmosine and isodesmosine) throughout the tissue (Sims and Bailey, 1981; Asghar, 1985; Greaser, 1997). The extent and stability of the crosslinks in elastin, even in mature tissues, is far greater than that found in collagen and would be unaffected by cooking (Sims and Bailey, 1981).

## **Analytical Methods for Quantitation of Meat Proteins**

The measurement of the amount of protein present in a sample becomes more complex as food scientists discover new and unusual protein materials and work with less characterized systems (Regenstein and Regenstein, 1984). Over 100 years, protein determinations have been performed on meat food products by procedures that measure the nitrogen content (converted to the equivalent protein content by an appropriate numerical factor) of the sample (Ellis, 1994). In the US, the legal basis by the government for determining protein for food products are based on the Kjeldahl nitrogen analysis, however, the test is slow (Regenstein and



Regenstein, 1984). Alternatively, King-Brink and Sebranek (1993) reported that combustion methods for protein analysis that release nitrogen at high temperatures and quantitate the nitrogen by thermal conductivity were shown to be a practical option to the classical Kjeldahl method.

### **Muscle Tissue**

In muscle tissue, different protein fractions have been isolated and quantitated. Helander, (1957) reported the optimal conditions for quantitative protein extraction from skeletal muscle. The author separated and quantitated different protein fractions by carefully washing and centrifuging the muscular tissue with solutions of increasing ionic strength. He found that the representative muscular tissue, contained about 60 percent of myofibrillar proteins, 35 percent of sarcoplasmic proteins and between 5 and 6 percent of stromal proteins.

Nuckles, et al. (1990) quantitated the protein distribution from selected pork and beef by-products. The authors extracted the low ionic strength soluble (LIS) proteins with 0.05 M Na phosphate buffer, pH 7.4. Likewise, high ionic strength soluble (HIS) proteins were extracted with 0.6 M NaCl, 0.05 M Na phosphate buffer, pH 7.4. The precipitate was designated insoluble proteins (IN). The protein content of each fraction was determined by the Kjeldahl procedure (AOAC).

### **Connective Tissue**

#### **Collagen**

Various methods for detection and estimation of collagen, based on chemical, physical, histochemical and immunological techniques, have been reported (Etherington and Sims,

1981; Bailey and Light, 1989). However, the selection of the analytical method for detection of collagen must be based on the type of tissue involved (Etherington and Sims, 1981).

Chemical methods have been used for some years for the quantitation of collagen content in meat, as in most other tissues. They are based on the determination of protein-bound hydroxyproline in the tissues. According to Bailey and Light (1989), the hydroxyproline content of the collagens varies from 13% by weight in Type I to 17.4% in Type III. Overall, a value of 14% is used as an approximation for muscle connective tissue. The chemical technique was described by Etherington and Sims (1981). Tissues to be analyzed are first acid-hydrolyzed to release the hydroxyproline from peptide linkage. The free amino acid then is determined colorimetrically after it is oxidized to pyrrole and reacted with Ehrlich's reagent (p-dimethyl-amino-benzaldehyde) to produce an intense red-brown color.

#### **Soluble / Insoluble Collagen**

In studies of meat functionality, determination of soluble and insoluble collagen could prove beneficial. Hill, (1966) described a procedure to determine the solubility of intramuscular collagen in meat animals of various ages. He solubilized the intramuscular collagen of muscles from cattle, sheep and pigs by heating in 1/4-strength Ringer's solution at 77°C for 1 hour. Hydroxyproline was determined by a modification of the Newmann and Logan method (1950).

#### **Collagen Types I and III**

Collagen Type III (reticulin) is normally associated with Type I collagen and is prominent in tissues which require a degree of flexibility. In meat, the ratio of Type I to III collagen is of interest because it may be correlated with taste panel perception of tenderness (Burson and Hunt, 1986). According to Bailey and Light (1989), estimation of Types I and III collagens in tissues can be made reasonably accurately by using the CNBr / SDS-polyacrylamide gel

technique. Although this method reveals only the ratio of Type I to Type III, it can be adapted for an approximate calculation of the absolute amount of each type.

Polyacrylamide gel electrophoresis (PAGE) is a widely used method for examining proteins and enzymes. Electrophoresis refers to the movement of charged colloidal particles and macromolecular ions under the influence of an electrical field (Pomeranz and Meloan, 1987). The velocity of movement depends on the molecule's net charge and frictional resistance (size and shape dependent) and on the strength of the electrical field applied (Regenstein and Regenstein, 1984). Sodium dodecyl sulfate (SDS) unfolds proteins, separates subunits and associates with the polypeptide backbone, lending a charge to the protein proportional to its molecular weight.

Burson and Hunt (1986) isolated intramuscular collagen containing endomysial and perimysial tissue from bovine skeletal muscle. Isolated intramuscular collagen was digested with cyanogen bromide and the proportion of Type I and III collagens determined from densitometric scans of peptides resolved by SDS-PAGE (9.5% acrylamide).

Swatland, (1987) suggested the use of a fluorometric method to estimate the connective tissue components of meat toughness (Type I and III collagen). He showed that fluorescence of Type I collagen was stronger than for Type III collagen over a range from 410 to 470 nm. A ratio of 510/440 nm was used to identify Types I and III collagen. This researcher found that the fluorescence emission spectrum of Type IV was similar to that of Type III collagen. The same was found for the fluorescence of elastin relative to that of Type I collagen.

### **Intramuscular Collagen (IMC)**

Fujii and Murota (1982) described a procedure for the isolation with high yield of collagen from human skeletal muscle without using protein denaturants. Fibrous material was recovered from a homogenate of skeletal muscle (0.1 M KCl, pH 7.2) through a series of

sequential extractions with Hasselbach-Schneider solution and 0.6 M KI - 0.06 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The recovery of collagen in the insoluble fraction was found to yield between 94.0 and 98.6% of the total collagen in muscle tissue. The high purity was confirmed by aminoacid analysis.

## Elastin

The quantitation of elastin in meat is performed by the destructive hydrolysis of all other insoluble materials. Elastin remains insoluble during hydrolysis and its original concentration in meat is back-calculated (Bailey and Light, 1989).

Bendall (1967) designed a method for determination of elastin from muscle tissue using numerous extractions with NaOH and various solvents. The procedure first removed fat, then collagen and muscle proteins together to leave a residue of elastin. He concluded that the elastin of various muscles of beef measured by this method was almost identical to that of purified elastin from ox *Ligamentum nuchae* (hydroxyproline values were less than 2% of dry weight).

Cross et al. (1973) reported that elastin isolated as the residue remaining after extraction with 0.1 N NaOH at 98°C for 45 min. yielded a material that was relatively homogeneous in composition and varied little with tissue source or animal age.

Bailey and Light (1989) described a technique using cyanogen bromide. Elastin was not solubilized by CNBr since it lacks methionine. The cyanogen bromide technique provided a simpler and quicker alternative to the lengthy and laborious method of hydrolysis in 0.1 N NaOH.

Zarkadas et al. (1988) developed a chromatographic method to quantitate the unique aminoacids that occur in proteins. The authors successfully applied the technique to determine the myofibrillar and connective tissue content of selected porcine skeletal muscles. Elastin content was calculated from the amounts of desmosine or isodesmosine present.

## **Functional Properties of Meat Proteins**

### **Meat Products**

The functional properties of proteins denote any physico-chemical property which affects the processing and behavior of protein in food systems, as judged by the quality attributes of the final product (Kinsella, 1976). In processed meats, the functional property of a protein depends on its aminoacid composition, molecular weight, solubility, thermal properties and the relationship of these properties to pH, temperature and salt concentration (Bailey and Light, 1989). Texture and appearance of these products are largely determined by the functional properties of their meat proteins.

In order to maximize the meat protein functional properties, meat processors reduce the particle size of meat tissues through comminution. Finely comminuted meat products are a mixture of proteins, fat particles, water, salt and often, carbohydrates (Barbut, 1995). The most important functional properties of meat proteins in comminuted products are solubility, emulsification capacity, gelation and water-binding (Kinsella, 1982).

### **Myofibrillar Proteins**

Myofibrillar proteins are recognized for their significant contribution to water and fat-binding and thus texture development in further-processed meats (Kenney, 1995). Production of a successful comminuted product with good water- and fat-binding and optimum texture, requires that myosin or actomyosin proteins be suspended-solubilized, denatured and then aggregated by heat to form a gel structure. The extend of solubilization depends on the ionic strength, pH, presence of ATP or added polyphosphates (Hamm, 1986). Common extraction procedures for myofibrillar proteins are conducted at ranges of ionic strength (determined by

salt) and pH of 0.6 to 1.3 and 5.8 to 6.2, respectively (Acton et al., 1983). Phosphates are added (up to 0.5 %) to aid in the solubilization of myosin. Tripoly- or pyrophosphates are not only effective in dissociating actomyosin (in postrigor meat) but also in increasing the pH and ionic strength (Whiting, 1988).

Currently, there are two theories that explain the interaction of protein-water (water holding capacity) and protein-lipid (fat holding capacity) associations in comminuted meat systems. Barbut (1995) explains in detail both theories. The emulsion theory states that fat is stabilized by the formation of an interfacial protein film around small fat globules. The film serves as an interface between the aqueous and the fat phases and prevents their coalescence. The physical entrapment theory emphasizes the fact that fat is entrapped within the protein matrix (before and during cooking). In both cases, the type of protein structure, or matrix, formed determines both the textural characteristics and water binding ability of meat products (Schmidt et al., 1981).

The matrix that provides texture and fat-holding properties to processed meats is the final result of thermally induced unfolding and aggregation (gelation) of muscle proteins (Foegeding, 1988). A successful gel is a balance between protein-water and protein-protein interactions (Whiting, 1987). According to Ferry (1948), heat-induced gelation involves the initial denaturation of native protein into uncoiled polypeptides, followed by aggregation of denatured proteins into a cross-linked gel network during heating. The heating regime used in thermal processing has a major effect on the textural properties of the final product (Foegeding, 1988). Acton and Dick (1984) distinguished gelation from coagulation, describing the latter as the random protein-protein interaction of denatured protein muscles. A slow aggregation step relative to denaturation favors a finer and more oriented gel network (Whiting, 1987). Trout and Schmidt (1987) indicated that water binding ability of cooked meat products is at a maximum at low temperature and progressively decreases at

temperatures above 55°C. They also indicated that heat-initiated increases in textural strength start at 40°C and increase progressively with temperature up to 80°C, then reach a plateau.

### **Sarcoplasmic Proteins**

Sarcoplasmic proteins in comminuted products are very important for the color (myoglobin) and to a lesser extent to texture (Barbut, 1995). MacFarlane et al. (1977) studied the binding of meat pieces by myosin, actomyosin and sarcoplasmic proteins. They found that at concentrations above 0.3 M NaCl, the addition of sarcoplasmic proteins to myosin proteins exerted a negative effect on the binding of meat pieces.

### **Stromal Proteins**

Stromal proteins which can be beneficial to meat batter stability, may also be detrimental to meat batter stability at high collagen levels (Ladwig et al., 1989). Whiting (1989) indicated that modest amounts of collagen improve the firmness and elasticity of comminuted meats. He also indicated that too much collagen can cause batter instability, resulting in moisture and fat losses during processing. Collagen shrinks and becomes granular at 60°C during thermal processing and, if a product contains excessive amounts of collagen, causes poor yields and texture (Whiting, 1988). When heated to 75°C to 80°C, collagen melts forming gelatin. The rate of gelatinization increases with temperature, occurring very rapidly at 125°C (Whiting, 1989).

### **Meat By-Products**

Few studies on the functional properties of meat by-products have been published. Knowledge of meat by-product protein functionality is important since these proteins are a good alternative to skeletal muscle protein. Because meat by-products contain rather different amounts of myofibrillar, sarcoplasmic and stromal proteins, it is necessary to understand how each group responds to temperature, ionic strength and pH.

Satterlee and Free (1973) studied the emulsifying capacity and stability of different high-protein powders obtained from beef (whole blood) and pork by-products (brain, duodenum, heart, liver, lung, kidney, spleen and stomach). They found that tissue powders which had a low emulsion capacity (spleen, heart and kidney) also produced sausages with low emulsion stability. The authors reported blood, lungs and stomach tissue powders as having emulsion capacity and stability comparable to that of lean beef. A correlation was established between the level of salt-soluble proteins and the emulsion capacity and stability of these by-products.

Oliveros et al. (1982) studied the physical and chemical characteristics of some selected beef by-products (tongue, esophagus, tripe, abomasum, small and large intestines). The authors found that all beef by-products had significantly lower protein content than lean beef. They reported that the majority of the by-products had less myofibrillar protein and more sarcoplasmic and stromal proteins than lean. They also reported that the stability of the different by-product emulsions (expressed as % cooked recovery) were similar to the stability of the emulsion made of lean beef, except for spleen. However, the authors observed that emulsions from spleen, liver, kidney and lung tended to have higher emulsion yields and smaller amounts of total liquid and solid loss than the other samples. They explained these differences in terms of the high water-holding capacity and low stromal protein content of these meat by-products. No numerical correlations were calculated for WHC and content of the different protein fractions.



A similar study with pork by-products (tongue, stomach, heart, lung, spleen, liver, kidney, small and large intestines) was conducted by Ibarra et al. (1985). They reported that the by-products (except for liver) had significantly lower crude protein content than pork lean. The authors found that the sarcoplasmic protein content of all the by-products except the heart was significantly higher than that of pork lean. As for the myofibrillar proteins of the different by-products, they found that these were all significantly lower than those of lean pork except the heart (similar to lean). In the case of the stromal proteins, they reported that all by-products were generally high except liver, kidney and spleen. The WHC results were difficult to interpret since they used the Carver press method and the "mushy" nature of some of the by-products (liver and spleen) resulted in zero values of free water. The authors, as in the above described study, did not identify the different proteins making up the three protein extracts. Likewise, no numerical correlations were calculated for % cooking recovery or WHC and the different protein fractions.

Another similar study with pork variety meats (liver, lung, stomach, spleen, heart, small and large intestine) was conducted by Kim et al. (1991). These authors found that the chemical composition of the by-products varied. The protein contents of the variety meats were less than that of lean meat and the extractability of water-soluble protein was higher than that of salt-soluble protein, which is different from lean meat. When the variety meats were heated to 60°C, a great deal of drip loss resulted from the stomach, heart, small and large intestine, whereas water absorption up to 12% occurred for liver, lung and spleen. Solubilities of proteins were higher at pH 7.0 than at pH 4.5 (both water- and salt-soluble proteins), however no statistical significance was reported. Lastly, they reported that functional properties of mixed variety meats were not cumulative and differed from that of individual variety meats.

Venegas et al. (1988) studied the functional properties of pork (stomach) and beef by-products (lungs, spleen, kidneys and testicles). The authors reported better water holding

capacity (WHC) for all meat by-products when compared to lean beef. A high WHC was attributed to the higher pH of the meat by-products as compared to lean beef.

McKeith et al. (1988) evaluated the composition, functional properties and appearance of surimi-like pork and beef by-products (hearts, weasand meat, head meat and tongues). Processing yield indicated that hearts were the most viable by-product for manufacture of surimi-like material. Protein concentration of the surimi-like material was higher than the original meat source used for processing. They found that surimi-like material from beef, pork and beef by-products had textural properties similar to or better than commercial fish surimi.

In a more recent study, Nuckles et al. (1990) investigated the composition and functional properties of selected meat by-products (pork lung lobes, pork liver, beef lung lobes, beef spleen and beef heart). They found that meat by-products varied in their proximate composition, amount of the three major protein fractions [low ionic strength soluble (LIS), high ionic strength soluble (HIS), and insoluble] and collagen content. Using a batter model system, they also found that reheat cook yield was positively correlated with the quantity of HIS proteins and the percentage myosin and actin in the HIS fraction, and negatively correlated with LIS proteins.

Kenney (1995) when commenting about the above described study by Nuckles et al. (1990) pointed out the significance of the proportion of collagen in the raw materials. He highlighted that those meat by-products with the lowest reheat yield had the lowest actin:myosin ratio as well as less myosin than either beef heart or mechanically separated chicken. He added that heating to 72°C was sufficient to hydrolyze or partially hydrolyze collagen to gelatin, which in turn could serve as a cold-set binder. However, when reheated (95°C, 10 min), the gelatin readily melted and was lost during the process, contributing to the decreased reheat yield.

From these studies it is clear that our current knowledge about meat by-product protein distribution and characteristics is not yet complete. More research is needed in order to better

characterize the different protein fractions (HIS, LIS and IN). More information is also needed on stability of proteins at higher temperatures (sterilization temperature), as well as the effect of various salts and pH on functionality characteristics such as water holding capacity and texture.

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**CHAPTER 3. PETFOOD: I. PHYSICO-CHEMICAL CHARACTERISTICS OF  
DIFFERENT MEAT BY-PRODUCTS AS COMPARED TO  
MECHANICALLY SEPARATED CHICKEN  
(MSC)**

A paper to be submitted to Meat Science

José Antonio Rivera<sup>1</sup>, Joseph G. Sebranek<sup>2</sup>, Robert E. Rust<sup>3</sup> & Louisa B. Tabatabai<sup>4</sup>

**ABSTRACT**

Pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and mechanically separated chicken (MSC) were evaluated for proximate composition, protein distribution and connective tissue. Proximate composition varied among meat by-products and MSC. Pork by-products contained the highest level of crude protein ( $p < 0.05$ ). Low levels of high ionic strength soluble (HIS) protein were obtained from meat by-products. Pork lungs and chicken viscera contained the highest levels of insoluble (IN) proteins ( $p < 0.05$ ). Total collagen values were positively correlated to IN proteins, intramuscular collagen (IMC) and elastin. No Types I and III collagen were detected by SDS-PAGE (12.5%) for the different meat by-products.

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<sup>1</sup> Graduate student, Department of Animal Science, Iowa State University. Primary researcher and author.

<sup>2</sup> Professor, Departments of Food Science and Human Nutrition and of Animal Science, Iowa State University. Corresponding author.

<sup>3</sup> Emeritus Professor. Department of Animal Science. Iowa State University.

<sup>4</sup> Professor. National Animal Disease Center, USDA, ARS, Ames, Iowa.

## INTRODUCTION

Raw materials of animal origin that are not suitable for human consumption are referred to as meat by-products (Flachat, 1978). Meat by-products are underutilized and low priced because they are regarded as inferior protein sources compared to skeletal muscle (Oliveros et al., 1982). A large percentage of this potentially valuable protein source is presently discarded because of the unaesthetic appearance of the material (Webster, 1982). Alternatively, meat by-products are processed into large quantities of animal proteins to be used by the feed industry (Fahey and Hussein, 1997). The petfood industry relies heavily on by-products of the meat packing and poultry processing industries as sources of protein (Morris, 1990).

Despite the wide use of meat by-products by the petfood industry, there have been very few attempts to characterize them. Most of the published research has concentrated on composition and quantitation of the different protein fractions (myofibrillar, sarcoplasmic and stromal) of selected beef and pork by-products (Oliveros et al., 1982; Ibarra et al., 1985; Kim et al., 1991). More recently, Nuckles et al. (1990) calculated the myosin and actin molar ratios of mechanically separated chicken (MSC) and selected pork and beef by-products. However, in all these studies few attempts were made to further characterize the connective tissue.

Meat by-products contain varying amounts of connective tissue (Bailey and Light, 1989a). Collagen and elastin are two of the major protein components of connective tissue (Jones, 1984). The distribution of these two components varies depending on their specific biological function. Collagen Type I makes up the largest proportion in muscle (Eyre, 1980). Collagen Type III is normally associated with Type I collagen and is prominent in tissues which require a degree of flexibility (Bailey and Light, 1989b).

The role and importance of connective tissue has been long recognized by the meat industry. In comminuted meat products, collagen does not bind proteins well and dilutes the

beneficial effects of the myofibrillar proteins (Whiting, 1989). According to Wiley et al. (1979) the use of "high collagen meats" must be limited to 15% of the total meat block when manufacturing comminuted products. Jones (1984) reported that collagen content affects the yield, texture and stability of meat products.

Although the petfood industry has a wide selection of protein sources of animal origin, the recent controversy surrounding the meat industry with bovine spongiform encephalopathy (BSE) has prompted the petfood industry to consider other sources of meat by-products such as poultry and pork by-products. Therefore, pork lungs, pork kidneys and chicken viscera were selected for this study. The objective of this study was to characterize these by-products in comparison with MSC with particular emphasis on the characterization of connective tissue. MSC has been extensively characterized and currently provides the meat industry with a standardized source of animal protein.

## **MATERIALS AND METHODS**

### **Materials**

Pork by-products (lung lobes, kidneys) from 45 normal slaughter weight barrows and gilts were obtained on batches of 15 hogs (designated as replicates) on three different slaughter days from the meat laboratory at Iowa State University. Pork by-products were collected during evisceration and chilled for 3 h at 4°C prior to grinding. Chilled by-products from different animals were combined and sequentially ground through 10 and 5 mm plates with a Biro grinder (Model 8, Marblehead, OH), hand mixed, vacuum packaged in bags of 0.7-1.0 Kg, frozen and stored at -23°C. Ground chilled chicken viscera (heads, feet and viscera) was obtained from Pilgrim's Pride Fresh Pet Food Division (De Queen, AR) and shipped to the Iowa State University meat laboratory in three 10 Kg batches from three

different production days. Upon arrival, the chicken viscera was vacuum packaged in plastic bags of 0.5 Kg, frozen and stored at  $-23^{\circ}\text{C}$ . Frozen MSC prepared from front chicken half-frames, breasts or rib cages was obtained from International Dehydrated Foods, Inc. (Monett, MO) and shipped to the Iowa State University meat laboratory in 20 Kg batches from three different production lots. At arrival, MSC was vacuum packaged in plastic bags of 0.5 Kg and stored at  $-23^{\circ}\text{C}$ . Meat samples were randomly selected and used as needed.

### **Proximate Analysis and pH**

Meat by-products and MDCM were analyzed for moisture, fat and ash according to AOAC (1990) procedures. Protein was measured by a nitrogen analyzer (Model FP-428, LECO Corporation, St. Joseph, MI). The pH was determined by blending 10 g of meat sample with 100 g of distilled water in a Waring Blender (Model 7010, New Hartford, CT) for 30 sec and measured using an Accumet pH meter (Model 925 pH/ion meter, Fischer Scientific Co., Pittsburgh, PA). All analyses were determined in triplicates.

### **Determination of Protein Fractions**

Nuckles et al. (1990) procedure was used to quantitate the low ionic strength soluble (LIS), high ionic strength soluble (HIS) and insoluble (IN) protein fractions. Samples of meat were removed from frozen storage and thawed at  $4^{\circ}\text{C}$  overnight. Meat samples were ground (Food Processor, Model 14081, Sunbeam Appliance Co., France) for 30 s. A weighed portion of meat was blended (Waring Blender, Model 7010, New Hartford, CT) for 1 min with 4 volumes of 0.05 M Na phosphate buffer, pH 7.4, extracted through constant stirring for 3 h at  $4^{\circ}\text{C}$  and re-extracted for 1 h following centrifugation at  $23,000\times g$  for 15 min (Model J-21C, Beckmen Instruments, Inc., St. Louis, MI). The supernatants containing the 0.05 M Na

phosphate soluble proteins from two extractions were combined and designated as LIS proteins. The precipitate was mixed with 4 volumes of 0.6 M NaCl, 0.05 M Na phosphate buffer, pH 7.4 and extracted twice as described previously. The supernatants were combined and designated as HIS proteins. The precipitate was designated as IN proteins. The protein content of each fraction was determined by a nitrogen analyzer (Model FP-428, LECO Corporation, St. Joseph, MI). The percentage of protein in each fraction was calculated by dividing the weight of total protein in each fraction by the weight of total protein in the meat sample. In the chicken viscera sample, an appreciable amount of bone chips was detected in the IN protein fraction. Therefore, the total IN protein fraction weight was adjusted for bone chips by a factor of 0.9944. This factor estimated that approximately 0.56% of the total chicken viscera sample weight was due to bone chips.

### **Collagen Determination**

Collagen was determined in duplicate on each meat by-product and MSC. Soluble and insoluble fractions were separated by the procedure of Hill (1966). Spectrophotometric determination of hydroxyproline in both fractions was performed according to Bergmann and Loxley (1963). Conversion factor used for both fractions was 7.25 (Goll et al., 1963). Collagen values were expressed in mg collagen/g of total sample.

### **Elastin Determination**

Meat by-products and MSC elastin content was determined in triplicate by the procedure outlined by Cross et al. (1973). A weighed portion of meat was placed in a 50 mL capped centrifuge tube to be extracted three times in a low-speed mechanical shaker (Model 50, Precision, Chicago, IL) at 25°C with 1.1 M potassium iodide plus 0.1 M potassium

phosphate buffer, pH 7.4 (10 mL/g of wet tissue) for 3.3, 2 h respectively and centrifuged (Model J-21C, Beckmen Instruments, Inc. St. Louis, MI) between extractions for 10 min at 1400X g. The tissue was washed twice with distilled water and the residue was recovered with low-speed centrifugation (1400X g). An excess of 2:1 chloroform-methanol was added to the residue and agitated in a low-speed mechanical shaker at 4°C for 2 h. Following extraction, the tissue was washed three times with distilled water and hydrolyzed in 0.1 N NaOH (10 mL/g of wet tissue) at 98°C for 50 min. The residue was washed with distilled water, centrifuged for 10 min at 1400X g, dried for 24 h at 102°C, cooled and weighed. Elastin content was reported as mg/g on a whole tissue basis (WTB). Elastin content in chicken viscera was adjusted for bone chips by a factor of 0.9944.

### **Intramuscular Collagen Determination**

Intramuscular collagen (IMC) was isolated using the procedure by Fujii and Murota (1982). IMC was subsequently homogenized (Model PT 10/35, Kinnematica, Switzerland) in distilled water and washed in a solution of 2% (w/v) sodium dodecyl sulfate (SDS) as described by Laurent et al. (1981). Lastly, IMC was rinsed with distilled water, lyophilized (Model 15 SRC-X, Virtix, Gardiner, N.Y) and stored desiccated at 4°C for not more of 90 days as suggested by Burson et al. (1986). IMC was expressed as dry weight in mg/g muscle. IMC was adjusted for bone chip content in chicken viscera by a factor of 0.9944.

### **SDS Gel Electrophoresis**

IMC samples were digested with cyanogen bromide (CNBr) and resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a slightly modified procedure by Burson et al. (1986). SDS-PAGE was done on a mini-protein apparatus (Model SE 400,



Hoeffer Scientific Instruments, San Francisco, CA) run through 1.0 mm slab gels containing 12.5% (w/v) acrylamide, 0.3% (w/v) bisacrylamide with a 4% acrylamide stacking gel, 0.2% (w/v) SDS. Gels were stained with 0.125% coomassie Brilliant Blue R250 (Bio-Rad Laboratories, Richmond, CA), 25% (v/v) isopropanol and 10% (v/v) acetic acid for 4 h and destained overnight with 25% (v/v) isopropanol and 10% (v/v) acetic acid at room temperature. Molecular weights were estimated by comparing mobilities of protein in the samples with mobilities of a high molecular weight standard mixture (Sigma Chem. Co., St. Louis, MO) and against purified Type I collagen standard from calf skin (Sigma Chem. Co., St. Louis, MO).

### **Statistical Analysis**

Means, standard errors and analysis of variance were determined by using the Statistical Analysis System (SAS Institute, Inc., 1991). The difference between means was determined by the least significant difference ( $p < 0.05$ ).

## **RESULTS AND DISCUSSION**

Proximate composition of meat by-products and MSC is tabulated in Table 1. The protein content of the meat by-products was significantly higher than MSC ( $p < 0.05$ ), except for chicken viscera. No meaningful difference was found between pork lung and kidney for levels of protein ( $p > 0.05$ ). Moisture content values were significantly higher for pork lungs and kidneys when compared to chicken viscera and MSC ( $p < 0.05$ ). The calculated protein:moisture ratios for pork lungs, pork kidneys, chicken viscera and MSC were 0.21, 0.21, 0.16 and 0.20 respectively. Fat content was greater for chicken viscera and MSC when compared to pork lungs and kidneys ( $p < 0.05$ ). However, no significant differences were found

within both groups ( $p>0.05$ ). Ash levels were not different from MSC ( $p>0.05$ ), except for pork lungs with a slightly lower level of ash. High levels of ash were expected with chicken viscera given the anatomic parts used in the processing of this by-product. The pH levels of the meat by-products differed significantly when compared to MSC ( $p<0.05$ ), with the exception of pork kidneys. A considerable amount of blood in pork lungs may have contributed to raising the pH. However, no meaningful differences were found between pork kidneys and lungs ( $p>0.05$ ). In chicken viscera, a lower pH value than MSC was expected. A low pH in chicken viscera could be the result of high microbial activity. Proximate composition values for pork lungs and kidneys were consistent to those reported in the literature (Ibarra et al., 1985; Nuckles et al., 1990; Kim et al., 1991). No published references were found for chicken viscera. Protein content for MSC was significantly lower compared to the 17.4% reported by Nuckles et al. (1990).

The protein fraction values for the different meat by-products are given in Table 2. The high ionic strength soluble proteins (HIS) for MSC were significantly lower than for chicken viscera ( $p<0.05$ ). No meaningful differences in values of HIS proteins were found among different meat by-products ( $p>0.05$ ). Low ionic strength soluble proteins (LIS) in MSC and pork kidneys were significantly higher than pork lungs and chicken viscera ( $p<0.05$ ). Pork lungs and chicken viscera contained the highest level of insoluble proteins (IN). Overall, the recovery of protein in MSC and meat by-products was higher for LIS and IN. Kim et al. (1991) reported that for different pork variety meats, the extractability of water-soluble proteins was higher than that of salt-soluble proteins. Likewise, values published by Nuckles et al. (1990) for the three different protein fractions for pork lungs are also consistent with our results, except for MSC. Some discrepancies with the protein fraction values were expected given the different origin of the MSC used by the researchers. Table 4 shows correlation coefficients for HIS, LIS and IN proteins. In general, less HIS proteins

were extracted at the gain of LIS proteins ( $r = -0.58$ ). Conversely, more IN proteins were obtained at the expense of LIS proteins ( $r = -0.94$ ).

Collagen composition of meat by-products and MSC is shown in Table 3. MSC contained the lowest level ( $p < 0.05$ ) of total collagen when compared to the meat by-products, except for kidneys. Comparable results were published by Nuckles et al. (1990) for MSC and pork lungs. However, the values reported by Nuckles et al. (1990) were significantly lower than ours. Chicken viscera contained the highest level of soluble collagen among the meat by-products and MSC ( $p < 0.05$ ). Conversely, chicken viscera contained the lowest level of insoluble collagen among the meat by-products, including MSC ( $p < 0.05$ ). Overall, the amount of insoluble collagen was greater ( $p < 0.05$ ) than soluble collagen for the meat by-products and MSC, except for chicken viscera. Total collagen values were strongly correlated ( $p < 0.05$ ) to IN proteins, IMC and elastin (Table 4). Insoluble collagen was positively correlated to IMC ( $r = 0.77$ ).

Elastin values (Table 3) were not significantly different among the meat by-products and MSC ( $p > 0.05$ ). Although not significant, pork lungs tended to have a high level of elastin. Bailey and Light (1989a) reported elastin as a major constituent of certain ligaments (40-60%) and other compliant tissues as lung and skin (2-5%). Elastin values were positively correlated ( $p < 0.05$ ) to IN proteins and IMC (Table 4).

Intramuscular collagen (IMC) values are shown in Table 3. The term IMC was first given by researchers Fujii and Murota (1982) to the insoluble fraction recovered from a homogenate of skeletal muscle. In this study, the term IMC refers to the insoluble fraction recovered from meat by-product and MSC homogenates. Meat by-product intramuscular collagen values were significantly higher than MSC ( $p < 0.05$ ). Among the meat by-products, pork lungs contained the highest level of IMC ( $p < 0.05$ ). A significant correlation ( $p < 0.05$ ) between IMC and IN proteins was found (Table 4). Such correlation was expected since both

procedures rely extensively on exhaustive water and salt-soluble protein extractions to obtain the residual insoluble fraction.

SDS-PAGE was performed to evaluate the proportion of Types I and III collagen in the connective tissue of the different meat by-products. In vertebrates, Types I and III collagen are the most abundant collagens and are found in almost all body tissues (Laurent et al., 1981). In meat, the ratio of type I to III collagen is of interest because it may be correlated with the taste panel perception of tenderness (Burson and Hunt, 1986). A SDS-PAGE (12.5 %) electrophoretogram is shown in Figure 1. Preliminary trials with 9.5 % (w/v) acrylamide, as suggested by Burson and Hunt (1986), did not show a clear band resolution. Molecular weights and purified Type I collagen served initially as standards (lanes 1 and 3-4, respectively). However, usage of a purified Type III collagen standard was unnecessary since no major bands appeared in lanes 5-6, 7-8 and 9-10 (pork lungs, pork kidneys and chicken viscera respectively).

## CONCLUSIONS

These results suggest that significant differences in functional properties can be expected among meat by-products and MSC. Low levels of HIS proteins among meat by-products may lead to poor water and fat binding when used in petfood formulations. Furthermore, high levels of LIS and IN proteins among meat by-products could also modify significantly the texture of manufactured petfood. Therefore, more research is needed to evaluate the functional properties of pork by-products (lung lobes, kidneys), chicken viscera and MSC in a high-moisture petfood model system.

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Table 1. Proximate composition of meat by-products and MSC.

Sample <sup>z</sup>	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	pH
Pork Lungs	16.57 <sup>a</sup>	79.15 <sup>a</sup>	2.10 <sup>b</sup>	1.10 <sup>b</sup>	7.19 <sup>a</sup>
Pork Kidneys	16.25 <sup>a</sup>	77.74 <sup>a</sup>	4.05 <sup>b</sup>	1.21 <sup>a</sup>	7.06 <sup>ab</sup>
Chicken Viscera	11.22 <sup>c</sup>	69.64 <sup>b</sup>	16.93 <sup>a</sup>	1.76 <sup>a</sup>	6.32 <sup>c</sup>
MSC	13.93 <sup>b</sup>	69.12 <sup>b</sup>	15.18 <sup>a</sup>	1.16 <sup>a</sup>	6.83 <sup>b</sup>
SEM	0.36	0.37	0.52	0.14	0.06

abc Means followed by the identical letters in the same column are not different ( $P < 0.05$ )

z Abbreviation used: MSC= Mechanically separated chicken.

Table 2. Protein fractions as a percentage of total protein of the high ionic strength soluble (HIS), low ionic strength soluble (LIS), and insoluble (IN) protein fractions of meat by-products and MSC.

Sample <sup>z</sup>	HIS Proteins (%)	LIS Proteins (%)	IN Proteins (%)
Pork Lungs	10.54 <sup>ab</sup>	48.38 <sup>b</sup>	41.07 <sup>a</sup>
Pork Kidneys	9.87 <sup>ab</sup>	66.65 <sup>a</sup>	23.48 <sup>bc</sup>
Chicken Viscera	14.81 <sup>a</sup>	53.18 <sup>b</sup>	32.01 <sup>ab</sup>
MSC	6.27 <sup>b</sup>	75.70 <sup>a</sup>	18.03 <sup>c</sup>
SEM	1.47	3.17	3.01

abc Means followed by the identical letters in the same column are not different ( $P < 0.05$ )

z Abbreviation used: MSC= Mechanically separated chicken.



Table 3. Collagen composition of meat by-products and MSC.

Sample	Total Collagen (mg/g meat)	Soluble Collagen (mg/g meat)	Insoluble Collagen (mg/g meat)	Elastin (%WTB)	IMC (mg/g meat)
Pork Lungs	25.79 <sup>a</sup>	1.91 <sup>b</sup>	23.88 <sup>a</sup>	1.77 <sup>a</sup>	79.90 <sup>a</sup>
Pork Kidneys	17.54 <sup>ab</sup>	2.27 <sup>b</sup>	15.27 <sup>b</sup>	1.19 <sup>a</sup>	36.93 <sup>b</sup>
Chicken Viscera	25.16 <sup>a</sup>	18.27 <sup>a</sup>	6.89 <sup>c</sup>	1.44 <sup>a</sup>	41.03 <sup>b</sup>
MSC	14.90 <sup>b</sup>	3.27 <sup>b</sup>	11.62 <sup>b</sup>	0.58 <sup>a</sup>	18.77 <sup>c</sup>
SEM	1.98	1.34	0.79	0.21	3.81

abc Means followed by the identical letters in the same column are not different ( $P < 0.05$ )

z Abbreviations used: MSC = Mechanically separated chicken, %WTB = %Wet tissue basis, IMC = Intramuscular collagen

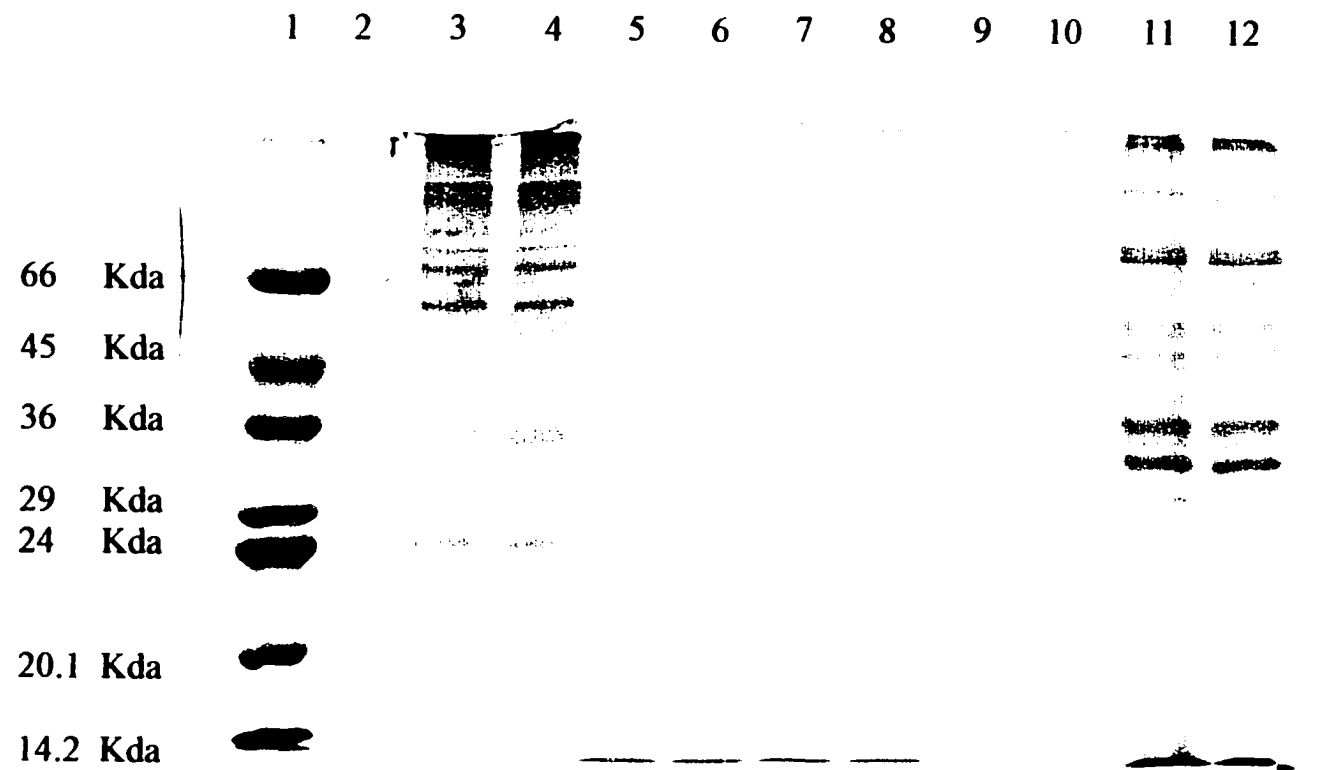
Table 4. Correlation coefficients among selected meat by-product and MSC physico-chemical values.

	TOTCOL	SOLCOL	INSCOL	ELAST	IMC	HIS	LIS
IN	0.82*	0.24	0.51	0.75*	0.87*	0.27	-0.94*
LIS	-0.86*	-0.39	-0.38	-0.80*	-0.82	-0.58*	
HIS	0.49	0.54	-0.15	0.45	0.25		
IMC	0.75*	-0.05	0.77*	0.74*			
ELAST	0.90*	0.36	0.46				
INSCOL	0.31	-0.62*					
SOLCOL	0.56						

z Correlation coefficients followed by \* were significant at ( $p < 0.05$ )

y Abbreviations used: MSC = Mechanically separated chicken, TOTCOL = Total collagen, SOLCOL = Soluble collagen, INSCOL = Insoluble collagen, ELAST = Elastin, IMC = Intramuscular collagen, HIS = High ionic strength soluble proteins, LIS = Low ionic strength soluble proteins, and IN = Insoluble proteins

Figure 1. Electrophoretogram of cyanogen bromide peptides of intramuscular collagen isolated from different meat by-products: (1) molecular weight standards, (3-4) Type I collagen standard, (5-6) pork lungs, (7-8) pork kidneys, (9-10) chicken viscera and (11-12) mechanically separated chicken on sodium dodecyl sulfate-polyacrylamide gel (12.5%)



**CHAPTER 4. PETFOOD: II. FUNCTIONAL PROPERTIES OF MEAT BY-PRODUCTS AND MECHANICALLY SEPARATED CHICKEN (MSC) IN A HIGH-MOISTURE MODEL SYSTEM**

A paper to be submitted to Meat Science

José Antonio Rivera<sup>1</sup>, Joseph G. Sebranek<sup>2</sup> & Robert E. Rust<sup>3</sup>

**ABSTRACT**

Contributions to water retention capacity (% WRC) and texture changes were determined for pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and mechanically separated chicken (MSC) as affected by pH and various salts in a high-moisture model system. The % WRC for meat by-products and MSC was increased by increased pH (4.5 - 6.8). Pork lungs and MSC had the highest % WRC ( $p < 0.05$ ) among the meat by-products. Meat by-product % WRC was not significantly ( $p > 0.05$ ) affected by salt (2%), phosphate (0.3%) or NaOH (0.075%). Chicken viscera had the lowest ( $p < 0.05$ ) mean texture measurements among the meat by-products and MSC. Strong negative correlations ( $p < 0.05$ ) were obtained for texture with total collagen, soluble collagen and high ionic strength soluble (HIS) proteins.

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<sup>1</sup> Graduate student, Department of Animal Science, Iowa State University. Primary researcher and author.

<sup>2</sup> Professor, Departments of Food Science and Human Nutrition and of Animal Science, Iowa State University. Corresponding author.

<sup>3</sup> Emeritus Professor, Department of Animal Science, Iowa State University.

## INTRODUCTION

The adoption of pets in millions of homes has provided the impetus for the development of a sophisticated petfood industry. Petfood for cats and dogs can be classified by their water content and method of preservation in three basic forms: canned, semi-moist and dry (Anonymous, 1982). Canned petfood (75-85 % moisture) makes a very important contribution to the diet of cats and dogs (Rainbird, 1988). High-quality nutritionally-balanced canned (moist) products are usually higher in animal proteins and in animal fat but lower in carbohydrates than dry or semi-moist products (Kelly, 1996). The petfood industry relies heavily on by-products of the meat packing and poultry processing industries as sources of animal protein (Morris, 1990).

Meat by-products are processed into canned petfood through controlled size reduction treatment (cubes, bits or chunks), followed by mixing with non-meat ingredients (dry compounds, binders and processing aids), after which the cans are filled, closed and thermally processed (Hischke, 1975). Meat by-product type and quantity used by the petfood processor depends largely on nutritional value, cost, availability, existing regulations, marketing purposes and functionality.

Few studies have been published on meat by-product functionality. Satterlee et al. (1973) studied the emulsifying capacity and stability of different high protein powders obtained from beef and pork by-products. The authors found that powders from blood, lungs and stomach tissue had emulsion capacity and stability comparable to that of lean beef. A correlation was established between the level of salt soluble proteins and the emulsion capacity and stability of these by-products. Ibarra et al. (1985) and Kim et al. (1991) studied the functional properties of different pork by-products. Venegas et al. (1988) and Nuckles et al. (1990) reported on the functional properties of selected beef and pork by-products.

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McKeith et al. (1988) found that surimi-like material from beef, pork and by-products had textural properties similar to or better than commercial fish surimi.

Information on the functional properties of meat by-products as it pertains to petfood is needed. Conceivable differences in meat by-product protein functionality can be expected in canned (moist) systems. Meat by-products contain varying amounts of myofibrillar proteins and high levels of sarcoplasmic and stromal proteins when compared to those of mechanically separated chicken (MSC) and lean meat (Rivera et al. 1998; Kim et al., 1991; Nuckles et al., 1990). The behavior of these protein fractions upon heating at sterilizing temperature (121°C) as well as the role of various salts and pH on water holding capacity and texture is poorly understood. Thus, the objectives of this research were: (1) to study the effects of inorganic phosphates, sodium hydroxide and sodium chloride on pH, water retention capacity (% WRC) and texture of selected meat by-products and MSC, and (2), to relate the physico-chemical properties for different meat by-products and MSC to % WRC and texture.

## **MATERIALS AND METHODS**

### **Materials**

Chemical-grade sodium chloride and sodium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ). A commercial blend of inorganic phosphates (sodium tripolyphosphate, sodium polyphosphate and sodium phosphate glossy) was obtained from Rhone-Poulenc (Cranbury, NJ). Pork by-products (lung lobes, kidneys) from 45 normal slaughter weight barrows and gilts were obtained on batches of 15 hogs (designated as replicates) on three different slaughter days from the Meat Laboratory at Iowa State University. Pork by-products were handled and packaged as described by Rivera et al. (1998). Ground, chilled chicken viscera (heads, feet and viscera) was shipped by Pilgrim's Pride Fresh

Pet Food Division (De Queen, AR) to the Meat Laboratory at Iowa State University from three different production lots. Frozen MSC prepared from front chicken half-frames, breasts or rib cages was obtained from International Dehydrated Foods, Inc. (Monnet, MO). MSC was shipped to the Meat Laboratory at Iowa State University from three different production lots. Upon arrival, the chicken viscera and MSC were vacuum packaged in 0.5 Kg plastic bags. Meat by-products and MSC were stored at -23°C. Raw materials were randomly selected and used as needed.

### **I. Effect of pH on water retention capacity (% WRC) of different meat by-products and MSC.**

To study the effect of pH on water retention capacity, meat by-products and MSC were homogenized for 30 sec in a Robot Coupe food processor (Model, R301 ultra, Bourgogne, France). Meat by-products and MSC were adjusted to final pH targets of 4.5, 5.5, 6.5 and 7.5 by direct addition of 0.5 N HCl or 0.5 N NaOH in a KitchenAid mixer (Model KSM90, St. Joseph, MI) with 30 sec mixing intervals. When no pH adjustment was needed, distilled water was added to compensate for the water added with HCL or NaOH. Final pH was measured directly in the meat homogenate with a Hanna pH meter (Model HI 9025C, Singapore). Aluminum cans of 5.28 cm X 2.74 cm (Central States Can, Massillon, OH) were filled with 85 g portions of meat by-product and MSC homogenates, sealed with a Dixie can seamer (Model 23, Athens, GA) and sterilized in a Market Forge autoclave (Model STM-E, Everett, MA) to 121°C temperature for 1 h. Cans were chilled overnight at 4°C and equilibrated to 25°C for 2 h prior to water retention determination. Water retention was determined by inversion of can contents onto a fine mesh wire food-strainer for 10 min. Calculation of % water retention capacity was based on the average of triplicate observations according to the following equation:

$$\% \text{ Water retention capacity} = [1 - (\text{water loss (g)} / 85)] * 100$$

## **II. Effects of inorganic phosphates, sodium hydroxide and sodium chloride on pH, water retention capacity (% WRC) and texture of different meat by-products and MSC.**

A 2X2X2 factorial design was constructed to study the effects of non-meat ingredients on pH, % WRC and product texture. A total of 8 treatments for each meat by-product and MSC were formulated. A weighed portion of each meat by-product and MSC was mixed with its appropriate levels of inorganic phosphates (0, 0.3 %), sodium hydroxide (0, 0.075 %) and sodium chloride (0, 2 %) in a Robot Coupe Food Processor (Model R301 ultra, Burgogne, France) for 1 min. The maximum amount of added sodium hydroxide was calculated using the ratio 1:4 of sodium hydroxide:phosphates as specified by the US Department of Agriculture (CFR, 1994). Final pH was measured directly in the meat homogenate with a Hanna pH meter (Model HI 9025C, Singapore) following mixing. Cans (5.28 cm X 2.74 cm) were manually filled with portions of 85 g, sealed without vacuum and sterilized in a Dixie autoclave (Model RDTI-3, Athens, Georgia) for 1 h after reaching an internal temperature of 121°C. Triplicate samples were prepared for each treatment. Following thermal processing, cans for all meat by-products and MSC were showered with cold water and chilled overnight at 4°C. Prior to texture determination, cans were equilibrated for 2 h at 25°C. Texture was determined with an Instron (Model 4502, Instron Corp., Canton, MA) equipped with a 1 kN load cell and star probe. Cross head speed was held constant at 500 mm/min. Measurements per can were recorded by compressing the content 80% of its original height in three separate locations following a triangular pattern. Meat by-product and MSC texture was expressed as the average maximum force ( $\text{Kg/m}^2$ ) recorded during penetration. After texture determination, cans were evaluated for % water retention capacity as previously described in part I.



## Statistical Analysis

Means, standard errors and analysis of variance were determined by using the Statistical Analysis System (SAS Institute, Inc., 1991). The difference between means was determined by the least significant difference ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### I. Effect of pH on water retention capacity (% WRC) of different meat by-products and MSC.

Water retention capacity as affected by pH for the different meat by-products and MSC is shown in Figure 1. Means for % WRC of meat by-products and MSC were significantly affected by pH ( $p < 0.05$ ). Except for chicken viscera, % WRC increased as values of pH became more alkaline for pork by-products and MSC. Hamm (1986) observed that in skeletal muscle, addition of base above the isoelectric point raised the protein net charge, thus increasing the interfilamental space and allowing more water to be immobilized. For the meat by-products, the observed increase in % WRC may have resulted from a high protein solubility. Kim et al. (1991) reported that solubility of proteins was higher at pH 7.0 than at pH 4.5. WRC for chicken viscera decreased when pH was raised from 6.78 to 7.77. Rivera et al. (1998) found high levels of collagen in chicken viscera. It is likely that the collagen in chicken viscera may have reached its isoelectric point which is approximately 7.4. The hydration level of collagen is at a minimum in the pH range of 6-8 (Bailey and Light, 1989). Pork lungs had the highest % WRC among the meat by-products and MSC at all pH values ( $p < 0.05$ ). Similar results were reported by Satterlee et al. (1973) when formulating a meat emulsion by replacing non-fat dry milk with different by-product tissue powders. They

found the least amount of water separation with lung tissue powder. Ibarra et al. (1985) reported no significant difference in expressible water between pork lungs and pork kidneys. However, the authors determined expressible water using uncooked meat by-products.

## **II. Effects of inorganic phosphates, sodium hydroxide (NaOH) and sodium chloride on pH, water retention capacity (% WRC) and texture of different meat by-products and MSC.**

The pH values among meat by-products and MSC were significantly affected ( $p < 0.05$ ) by salt, phosphate and NaOH (Table 1) as expected. The pH was significantly different ( $p < 0.05$ ) among meat by-products and MSC regardless the level of salt, phosphate and NaOH added (Table 2). A slight but significant ( $p < 0.05$ ) decrease in pH was observed among meat by-products and MSC when salt was used (Table 2). Conversely, meat by-products and MSC pH levels were raised ( $p < 0.05$ ) with the addition of 0.075 % NaOH (Table 2). Phosphates increased the pH only when added to chicken viscera.

Water retention capacity for the different meat by-products and MSC is displayed in Table 3. Pork lungs and MSC had the highest % WRC ( $p < 0.05$ ) among the meat by-products. Meat by-products were not significantly improved ( $p > 0.05$ ) by added levels of salt, phosphate and NaOH (Table 3) for % WRC. Nuckles et al. (1990) found that model system frankfurters produced with by-products had significantly lower reheat yield ( $p < 0.05$ ). They reported that reheat yield was positively correlated with the quantity of (HIS) proteins and the percentage myosin and actin in the HIS protein fraction. Rivera et al. (1998) found low levels of HIS proteins among meat by-products (pork lungs, pork kidneys and chicken viscera) and MSC as compared to LIS and IN proteins. Nuckles et al. (1991) also found that substitution of LIS or IN proteins into HIS protein gels altered expressible moisture. Bailey and Light (1989) indicated that the water holding capacity of collagen is not greatly affected by NaCl. They

stated that polyphosphates tended to decrease the water holding of collagen in the pH range 5-8. Poulanne and Ruusunen (1981) indicated that pH and ionic strength do not directly affect collagen, but may improve the batter-forming ability of the myofibrillar protein. Addition of salt to MSC was very effective in improving the % WRC ( $P < 0.05$ ). Slight increases in % WRC for MSC was also observed with added NaOH ( $p < 0.05$ ). Although not significant, phosphates also tended to increase the % WRC for MSC (Tables 1 and 3). The different response by MSC to ionic strength and pH compared to the by-products probably resulted from its HIS protein quality and the level of IN proteins. Nuckles et al. (1990) calculated higher ( $p < 0.05$ ) percentages of myosin and actin for MSC and beef heart than other by-products. Rivera et al. (1998) reported lower levels of IN proteins for MSC when compared to meat by-products. % WRC for chicken viscera was higher than for pork kidney % WRC means, improving considerable over those reported in part I (i.e. 74.40-76.26 vs. 49.83-61.82). More water was added to chicken viscera in part I to adjust the levels of pH, thus having more excess water to bind.

Texture measurements in some meat by-products and MSC were significantly affected ( $p < 0.05$ ) by phosphates and NaOH (Table 1). Chicken viscera had the lowest ( $p < 0.05$ ) Instron peak means among the meat by-products and MSC, regardless of levels of phosphates and NaOH (Table 4). Mean measurements for pork lungs and MSC texture decreased significantly ( $p < 0.05$ ) with added phosphates and NaOH (Table 4). An interaction ( $p < 0.05$ ) for salt was observed for pork lungs (Table 1). Downward changes in Instron peak means can be attributed to the ability of pork lungs and MSC to bind water. Meullenet et al. (1994) evaluated effects of collagen and added water on texture of frankfurters using sensory evaluation and torsion tests. They reported that added water resulted in softer, less springy and juicier frankfurters. High values in texture for pork kidneys among the different meat by-products and MSC reflect not only its inherent inability to bind water (Table 3), but also the lack of effectiveness of salt and phosphates for this by-product. However, when NaOH was

added, a soft texture resulted (Table 4). Again, a softer texture may have resulted as a result of an increase in % WRC. Although not significant, %WRC of pork kidneys increased when the pH was increased (Tables 2 and 3). Venegas et al. (1988) studied whole offal homogenates (beef lung, spleen, kidney, testicle and pig stomach) and beef skeletal muscle for water holding capacity (WHC). They showed that the offal had significantly greater WHC due to its higher pH. Whiting (1989) indicated that modest quantities of collagen improve the firmness and elasticity of comminuted meats, however too much can lead to batter instability with the associated moisture and fat losses during thermal processing.

Correlation coefficients among meat by-product and MSC physico-chemical values (Rivera et al., 1988) and model system properties are shown in Table 5. Means for % WRC were positively correlated to insoluble collagen, intramuscular collagen and insoluble protein (IN) values. It is likely that these forms of collagen underwent partial solubilization during heating to form gelatin upon cooling, thus helping to bind some of the free water. Whiting (1989) pointed out that gelatin and denatured collagen are strong water binders. According to Bailey and Light (1989), the water holding capacity of collagen increases two-fold following thermal denaturation at 70°C. Moreover, the rate of gelatinization increases with temperature, occurring very rapidly at 125°C (Whiting, 1989). Two significant negative correlations were also obtained for texture measurements with total and soluble collagen ( $p < 0.05$ ). Severe heat treatment during sterilization most likely helped to solubilize collagen, thus resulting in a soft texture. Wiley et al. (1979) reported a significant negative correlation between the amount of soluble collagen and the water and fat-binding properties of 21 raw sausage materials. Lastly, a strong negative correlation was found between texture and the HIS proteins ( $p < 0.05$ ). Similar results were reported by Nuckles et al. (1990) using a frankfurter model system produced with by-products. They found that apparent shear stress and strain at failure of the model system were positively correlated with HIS proteins and the percentage of myosin and actin within the HIS fractions.

## CONCLUSIONS

Because %WRC of meat by-products was not improved by salt, phosphates or sodium hydroxide, these nonmeat ingredients may not be important to petfood systems with high levels of meat by-products. On the other hand, these nonmeat ingredients may be critical to maximizing functionality of the low levels of myofibrillar type proteins which may be included. The textural effects of collagen for softening the product mixtures should be useful in controlling overall product texture. Control of pH can have a direct effect on texture. A soft texture was attributed to the inherent ability of pork lungs and MSC to bind more water at high pH values. It is conceivable that improvements in texture (high Instron values) can be achieved by reducing the level of total collagen when mixing meat by-products with MSC. Combination of MSC and meat by-products in the presence of these salts need to be studied in further detail to determine the most effective use of ingredients.

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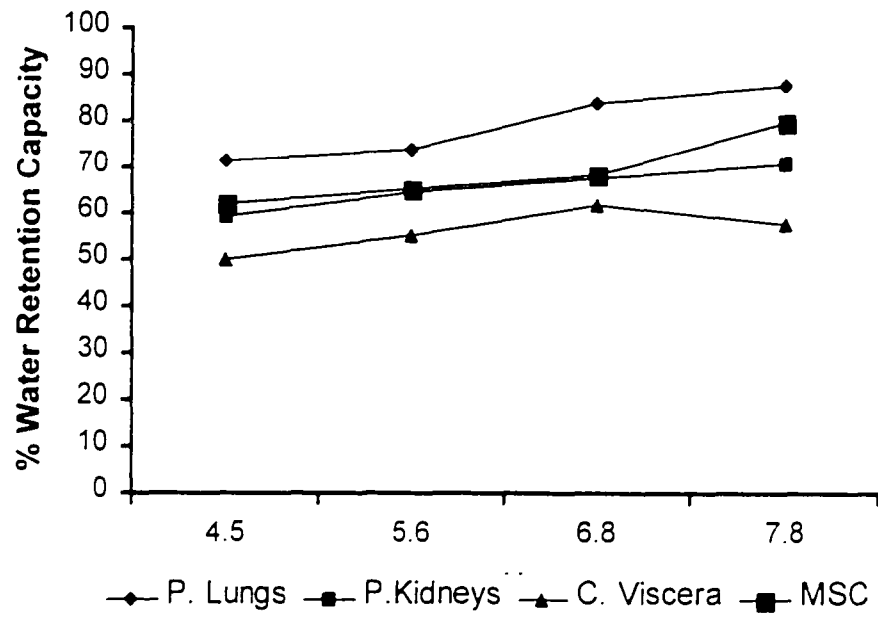


Figure 1. Effect of pH on % water retention capacity by different meat by-products and mechanically separated chicken (LSD = 5.83,  $p < 0.05$ ).



Table 1. Significant effects of meat source, % salt, % phosphate and % NaOH on final pH, % WRC and texture of canned products.

Main Effect	pH	%WRC	Texture
Sample (Meat source)	***	***	***
Salt	***	NS	NS
Sample*Salt	NS	***	**
Phosphate	*	NS	**
NaOH	***	*	***
Sample*NaOH	***	NS	NS

\*\*\* Significant at  $p < 0.001$

\*\* Significant at  $p < 0.01$

\* Significant at  $p < 0.05$

NS Non-significant at  $p < 0.05$

z Abbreviations used: % NaOH = % Sodium hydroxide, % WRC = % Water retention capacity

Table 2. Final pH of meat by-product and MSC mixtures.

% Salt	%Phosphate	%NaOH	pH			
			P.Lungs	P.Kidneys	C.Viscera	MSC
0			7.44 a*	7.11 b*	6.17 c*	6.90 d*
2			7.12 a*	6.81 b*	5.72 c*	6.58 d*
	0		7.24 a	6.95 b	5.87 c*	6.70 d
	0.3		7.31 a	6.96 b	6.02 c*	6.78 d
		0	6.83 a*	6.59 b*	5.85 c*	6.44 d*
		0.075	7.72 a*	7.32 b*	6.04 c*	7.04 d*

- z Means followed by the same letter in the same row are not significantly different at  $p < 0.05$ . The least significant difference for comparing meat by-products across a given level of a factor is 0.131.
- y Means not followed by the symbol \* in the same column within a given factor are not significantly different at  $p < 0.05$ . The least significant difference for comparing two levels of a factor within meat by-product is 0.131.
- x The pooled SEM for overall mean pH is 0.046.
- w Abbreviations used: P. Lungs = Pork lungs, P. Kidneys = Pork kidneys, C. Viscera = Chicken viscera, MSC = Mechanically separated chicken.

Table 3. Water retention capacity % of products with meat by-products and MSC.

			% WRC			
% Salt	%Phosphate	%NaOH	P.Lungs	P.Kidneys	C.Viscera	MSC
0			90.08 <sup>a</sup>	69.29 <sup>b</sup>	76.26 <sup>c</sup>	78.40 <sup>c*</sup>
2			89.52 <sup>a</sup>	68.57 <sup>b</sup>	74.40 <sup>c</sup>	88.00 <sup>a*</sup>
	0		90.05 <sup>a</sup>	68.27 <sup>b</sup>	74.43 <sup>c</sup>	81.17 <sup>d</sup>
	0.3		89.55 <sup>a</sup>	69.58 <sup>b</sup>	76.24 <sup>c</sup>	85.23 <sup>d</sup>
		0	88.73 <sup>a</sup>	67.22 <sup>b</sup>	74.93 <sup>c</sup>	81.10 <sup>d*</sup>
		0.075	90.87 <sup>a</sup>	70.64 <sup>b</sup>	75.73 <sup>c</sup>	85.30 <sup>d*</sup>

- z Means followed by the same letter in the same row are not significantly different at  $p < 0.05$ . The least significant difference for comparing meat by-products across a given level of a factor is 4.22.
- y Means not followed by the symbol \* in the same column within a given factor are not significantly different at  $p < 0.05$ . The least significant difference for comparing two levels of a factor within meat by-product is 4.22.
- x The pooled SEM for overall mean pH is 1.49.
- w Abbreviations used: P. Lungs = Pork lungs, P. Kidneys = Pork kidneys, C. Viscera = Chicken viscera, MSC = Mechanically separated chicken.

Table 4. Means for texture measurements of products with meat by-products and MSC.

% Salt	%Phosphate	%NaOH	Texture (Kg/m <sup>2</sup> )			
			P.Lungs	P.Kidneys	C.Viscera	MSC
0			3.95 a*	4.81 b	0.69 c	4.47 ab
2			2.67 a*	4.92 b	0.67 c	4.62 b
	0		3.69 a*	5.13 b	0.67 c	4.84 b*
	0.3		2.93 a*	4.60 b	0.69 c	4.25 b*
		0	3.87 a*	5.22 b*	0.67 c	4.89 b*
		0.075	2.75 a*	4.51 b*	0.70 c	4.20 b*

- z Means followed by the same letter in the same row are not significantly different at  $p < 0.05$ . The least significant difference for comparing meat by-products across a given level of a factor is 0.5831.
- y Means not followed by the symbol \* in the same column within a given factor are not significantly different at  $p < 0.05$ . The least significant difference for comparing two levels of a factor within meat by-product is 0.5831
- x The pooled SEM for overall mean pH is 0.21.
- w Abbreviations used: P. Lungs = Pork lungs, P. Kidneys = Pork kidneys, C. Viscera = Chicken viscera, MSC = Mechanically separated chicken.

Table 5. Correlation coefficients between meat by-product and MSC physico-chemical values and model system properties.

	PH	% WRC	Texture
Total Collagen	-0.0621	0.2867	-0.7181*
Soluble Collagen	-0.6771*	-0.2719	-0.8615*
Insoluble Collagen	0.6990*	0.5172*	0.4035*
Elastin	0.1705	0.1840	-0.4643*
Intramuscular Collagen	0.3875*	0.4796*	-0.2246
HIS Proteins	-0.3753*	-0.2582	-0.8219*
LIS Proteins	-0.0389	-0.2802	0.6252*
IN Proteins	0.1775	0.4358*	-0.4899*
Texture	0.3764*	-0.0545	
%WRC	0.3240		
pH			

z Correlation coefficients followed by \* were significant at ( $p < 0.05$ ).

y Abbreviation used: % WRC = % Water retention capacity, HIS = high ionic strength soluble proteins, LIS = low ionic strength soluble proteins and IN = insoluble proteins.

## CHAPTER 5. GENERAL SUMMARY

Three experiments were described in the present dissertation. Experiments were conducted to evaluate the physico-chemical characteristics and functional properties of selected pork by-products (lung lobes, kidneys) and chicken viscera (head, feet, viscera) in a high-moisture petfood model system. Mechanically separated chicken (MSC) served as control. Experiments were undertaken to better understand the effect of environmental factors (pH, ionic strength) and processing conditions (temperature) on final texture and water retention capacity (%WRC) of the selected meat by-products and MSC. Correlations were obtained to relate the functional properties of meat by-products and MSC to their individual physico-chemical characteristics.

Proximate composition values were found to vary significantly among meat by-products and MSC. Pork by-products contained the highest levels of protein and moisture as well as the lowest levels of fat when compared to chicken viscera and MSC. Overall, meat by-products and MSC contained higher levels of LIS and IN proteins than HIS proteins. The highest level of IN proteins were found in pork lungs and chicken viscera. Strong positive correlations were found for IN proteins against total collagen, elastin and IMC values. SDS-PAGE did not reveal appreciable amounts of Type I and III collagens for the different meat by-products. These results suggest that significant differences in functional properties can be expected among meat by-products and MSC. Low levels of HIS proteins among meat by-products may lead to poor water and fat binding when used in petfood formulations. Furthermore, high levels of LIS and IN proteins among meat by-products could also significantly modify the texture of manufactured petfood.

The pH value had a significant effect ( $p < 0.05$ ) on the % WRC of pork by-products (lung lobes, kidneys), chicken viscera (head, feet and viscera) and MSC. Pork lungs and MSC had the highest % WRC ( $p < 0.05$ ) among the meat by-products. Instron peak values contributed by pork lungs and MSC decreased significantly with the addition of phosphates (0.3%) and

NaOH (0.075%). A soft texture was attributed to the inherent ability of pork lungs and MSC to bind more water at high pH values. A significant negative correlation ( $p < 0.05$ ) was found for texture with total collagen, soluble collagen and HIS proteins. Instron values were affected more than %WRC by the use of inorganic salts.

Overall, %WRC was not improved ( $p > 0.05$ ) with the addition of inorganic salts to meat by-products while MSC was positively improved ( $p < 0.05$ ) with the addition of salt and sodium hydroxide. It is conceivable that addition of MSC to individual meat by-products could improve the %WRC when mixed with inorganic salts in canned petfood but the extent of improvement may depend on proportions used. Improvements in texture (high Instron values) could also perhaps be achieved by reducing the level of the total collagen when mixing meat by-products with MSC. Further research is needed to determine appropriate levels of MSC to be added to individual or composite (mixture of one or more) meat by-products in the formulation of canned petfood. Lastly, in addition to varying levels of connective tissue, levels of fat should also be investigated when formulating canned petfood.

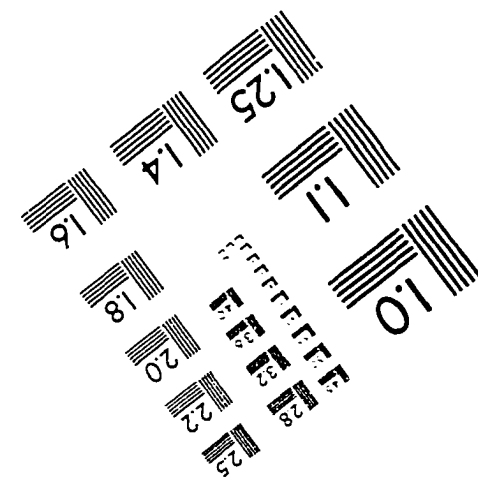
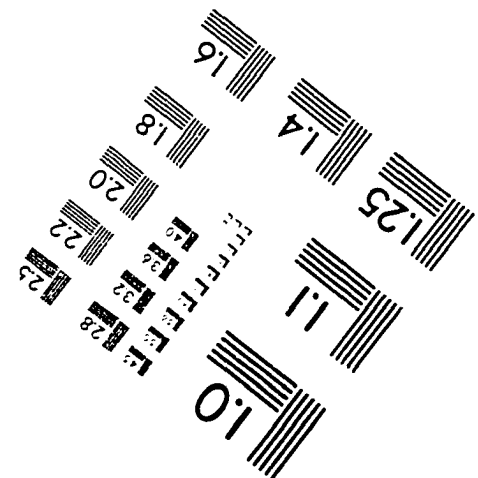
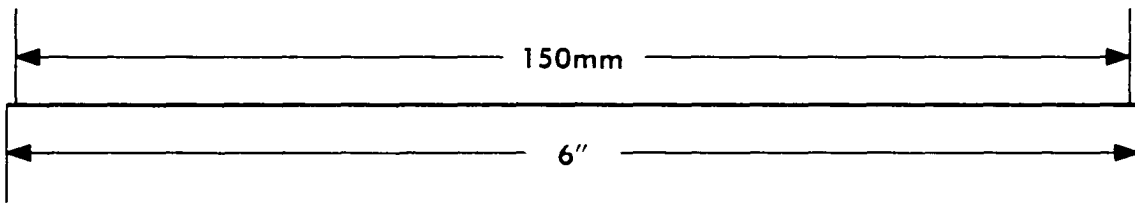
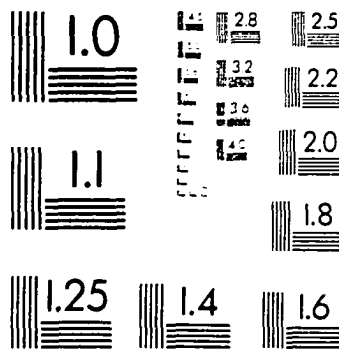
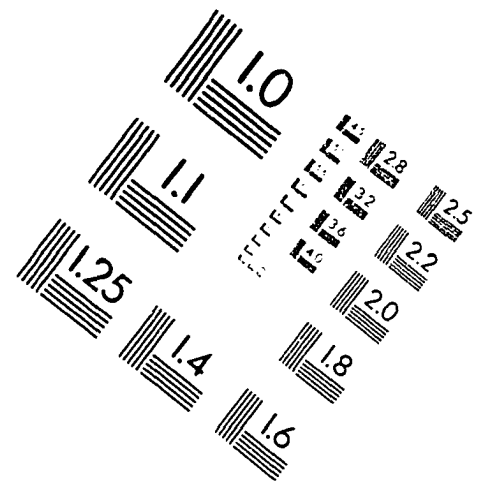
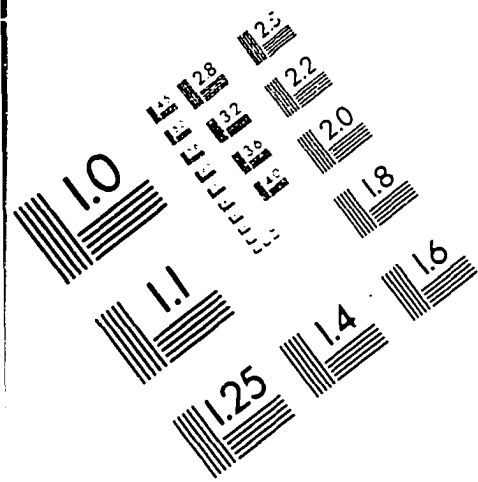
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1653 East Main Street  
Rochester, NY 14609 USA  
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